

## Genetics and Geography of Sex Determination in the Poeciliid Fish, *Xiphophorus maculatus*

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(Text-figure 1)

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### INTRODUCTION

OF THE more than 140 known species of poeciliid fishes, not more than two dozen have been studied in any detail, yet among these sex-determining mechanisms have been discovered that are unique for vertebrates (Gordon, 1947; C. Hubbs, 1964; Hubbs & Hubbs, 1932; Kallman, 1962; Miller & Schultz, 1959; Schröder, 1964; Schultz, 1961). One species, *Xiphophorus maculatus*, the southern platyfish, is thought to possess two sex-determining systems. In certain strains, the females are the homogametic sex ( $XX \text{ ♀}$ ,  $XY \text{ ♂}$ ), while in other strains the male is homogametic ( $WY \text{ ♀}$ ,  $YY \text{ ♂}$ ). *X. maculatus* lives in the Atlantic lowlands of Mexico, Guatemala and British Honduras. It ranges from the rivers of

British Honduras westward across the Petén district of Guatemala north to the Río Jamapa, near Veracruz, Mexico. It is absent from the Yucatan peninsula (Text-fig. 1).

The  $WY$ - $YY$  system of *X. maculatus* was independently discovered by Bellamy (1922, 1928) and Gordon (1927) and subsequently confirmed by Breider (1937, 1942) and Kosswig (1938). All four investigators worked with domesticated stocks that had been imported into Germany around the turn of the century (Gordon, 1927). The location in Central America from which the stocks originated was never recorded, although many years later Gordon (1952) was able to deduce that the Belize River was the likely place of origin. Gordon (1946, 1947, 1951 a, 1952) discovered that the platyfish from three Mexican rivers, the Ríos Jamapa, Papaloapan and Coatzacoalcos, possessed a different sex-determining mechanism ( $XX \text{ ♀}$ ,  $XY \text{ ♂}$ ), and that a commercial stock, allegedly from British Honduras, possessed the  $WY$ - $YY$  system. Gordon (1950 a, 1951 a, 1952, 1954, 1957) also briefly reported that females of the platyfish population of the Río Grijalva, Mexico, were homogametic, while those from the New and Belize Rivers in British Honduras were heterogametic. However, no details were published. Gordon suggested that the platyfish with opposing sex-determining mechanisms were geographically isolated and that fish living in rivers of British Honduras, which flow into the Caribbean, possess the  $WY$ - $YY$  system, while fish inhabiting rivers that drain into the Gulf of Mexico possess the  $XX$ - $XY$  type.

The  $XX$ - $XY$  sex-determining mechanism has also been found in *Xiphophorus variatus* by Bellamy (1936), Kosswig (1937) and Rust (1939) and in *X. milleri* by Kallman (1965). In other species, no sex-linked characters have ever been detected, but interspecific crosses indi-

cate that *X. couchianus* (Gordon & Smith, 1938; Gordon, 1946; Zander, 1962) and perhaps *X. montezumae cortezi* (Kosswig, 1959; Zander, 1965) have the  $XX$ - $XY$  system. The swordtail, *X. hellerii*, appears to have a polygenic sex-determining mechanism (Gordon, 1957; Kosswig, 1964; Peters, 1964).

Fish (*X. maculatus*) with different sex-determining systems are morphologically indistinguishable (Gordon & Gordon, 1954; Rosen, 1960) and mate readily not only with each other, but also with *X. variatus*. The offspring are fully fertile; the  $WY$ ,  $WX$  and  $XX$  genotypes generally resulting in females, the  $XY$  and  $YY$  conditions always in males, regardless from which populations or species the  $X$  or  $Y$  chromosomes have been derived (Atz, 1959; Bellamy, 1936; Gordon, 1951 a, 1952; Gordon & Smith, 1938; Kosswig, 1935; Öktay, 1959 a, 1962). Only a single cross in which the  $WY$  genotype differentiated into males has been recorded (Gordon, 1951 a). In this exceptional cross, the  $Y$  chromosome had been derived from the Río Coatzacoalcos population of *X. maculatus*. This cross has never been repeated and, therefore, it is too early to speculate whether the "Y" from this population has a stronger male determining potential than the  $Y$  chromosome from the other populations. In an attempt to determine more precisely the geographic distribution of the two mechanisms, *X. maculatus* were collected in several areas of Mexico, British Honduras and Guatemala from which they had never before been taken alive, and their sex chromosome constitution was analyzed in the laboratory.

#### MATERIAL AND METHODS

*Identification of Sex.* — In *X. maculatus*, as in all other poeciliid fishes, the sexes are readily distinguished by the shape of the anal fin. In males this transforms into an intromittant organ, the gonopodium, at the time of sexual maturity. In extremely rare cases a fish may develop without any gonad. Such fish superficially resemble females but possess a body shape different from that of either sex. In cases of doubt, however, sex was ascertained by autopsy. Although more than 100,000 platyfish have been raised at the Genetics Laboratory during the last 25 years, not a single female (fish with an ovary) has ever transformed into a male (fish with a testis) or developed a gonopodium. Consequently, in this paper a sex-reversed fish is one that is functionally one sex, but genotypically the other.

*Identification of Sex-determining Mechanism.* — Identification of the sex chromosome constitution of wild-caught *X. maculatus* is

greatly facilitated by the existence of (1) laboratory stocks of known sex chromosome constitution and geographic origin and (2) a series of phenotypically distinct, dominant multiple alleles at a sex-linked locus. The chromosome constitutions of the eight laboratory stocks are listed in Table 1. The origin, sex ratio and sex-linked pigment patterns of these strains are described below. Of the five sex-linked macromelanophore alleles that Gordon (1948) described from natural populations, four are involved in the crosses reported in this paper:

*N* — Nigra: irregular black blotches or bands along the flanks of the fish,

*Sr* — Stripe-sided: macromelanophores arranged in horizontal rows along the flanks,

*Sp* — Spot-sided: small, irregular spots of macromelanophores along the flanks of fish,

*Sd* — Spotted-dorsal: irregular spots of macromelanophores in the dorsal fin.

A difficulty occasionally encountered in recognizing these patterns is that the phenotypic expression of the macromelanophore gene may vary from zero penetrance to enhancement resulting in a mild melanosis. However, long experience with our reference stocks has enabled us to anticipate such difficulties. The *Sd* gene of *Jp-163 A* is only rarely expressed in inter-strain platyfish hybrids, for example. In the *Np* strain, the *N*, *Sr* and *Sd* alleles do not manifest themselves in a small percentage of fish. Some of the macromelanophore genes of the Lake Petén fish also exhibit low penetrance in certain hybrid combinations.

Diagnostic for the  $XX$ - $XY$  sex-determining mechanism are the following modes of inheritance:

Father to son (pigment gene located on  $Y$  chromosome).

Father to daughter (pigment gene located on  $X$  chromosome of father).

Maternal pigment pattern inherited by one-half of daughters and one-half of sons (mother heterozygous for pigment gene, located on  $X$  chromosome).

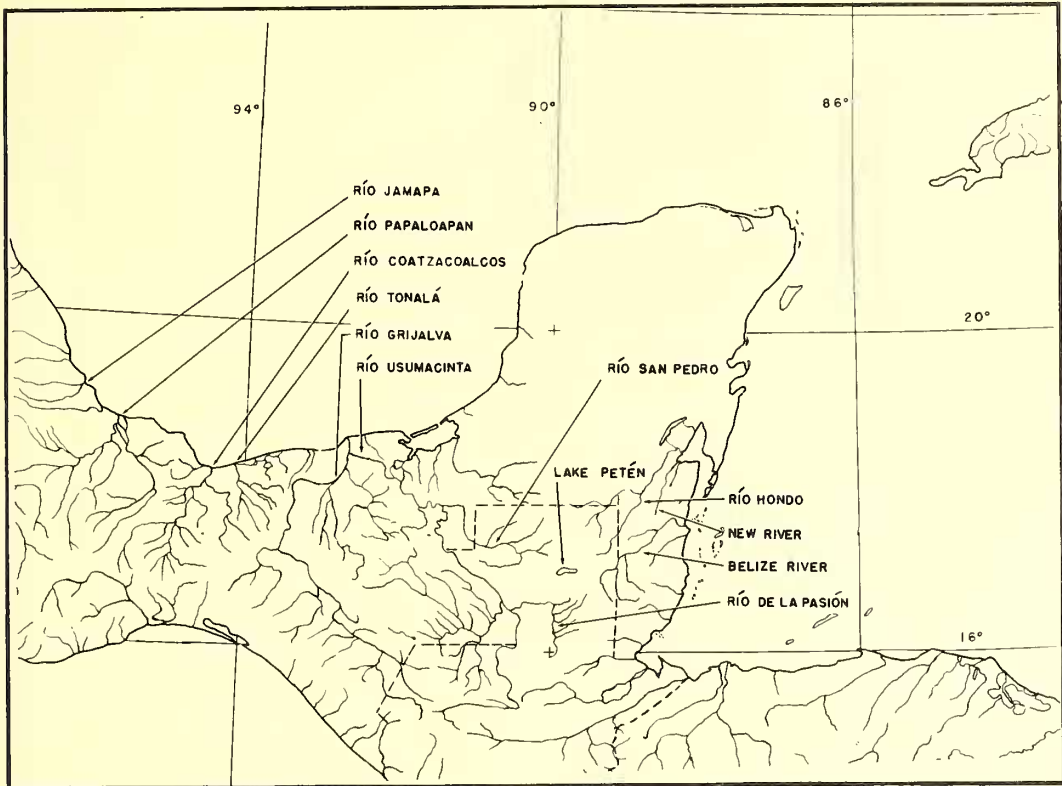
Indicative for the  $WY$ - $YY$  sex-determining system are the following modes of inheritance:

Mother to daughter (pigment gene located on  $W$  chromosome).

Mother to son (pigment gene on  $Y$  chromosome of mother).

Paternal pigment pattern inherited by one-half of daughters and one-half of sons (father heterozygous for pigment gene, located on  $Y$  chromosome).

The last three types of inheritance, although



TEXT-FIG. 1. Map of southern Mexico, Guatemala and British Honduras showing major river systems inhabited by the platyfish, *Xiphophorus maculatus*. Location of collecting stations: Belize — at mouth of Belize River; San Estevan — 25 km. inland from mouth of New River; Douglas and San Antonio — on Río Hondo, 32 and 40 km. inland (by air),

respectively; Tikal — 25 km. NNE of Lake Petén; Remate — at eastern tip of Lake Petén; Flores — near western end of Lake Petén; Carmelita — 55 km. NNW of Lake Petén, on tributary of Río San Pedro; Sebol — near the source of Río de la Pasión. Villahermosa is located at the tip of the arrow pointing to Río Grijalva.

consistent with the WY-YY system, cannot rule out the possibility that one of the parents carried an X chromosome; identical results are expected from the following three crosses:  $WY \times YY$ ,  $WX \times YY$ ,  $WY \times XY$ .  $WX$  and  $WY$  females can be distinguished by crossing them with  $XY$  males of the reference stocks in which the X and Y chromosomes are marked by different pigment genes.  $WY$  females give rise to two types of male offspring, while  $WX$  females give rise to only one class of sons. In addition the offspring of  $WX$  females occur in a 3:1 sex ratio.

In the absence of pigment markers,  $XX$  females can be distinguished from those carrying a  $W$  chromosome by mating them with  $YY$  males from reference stocks.  $XX$  females give rise to all-male offspring, while  $W$  females produce males and females. Similarly,  $XY$  males can be distinguished from  $YY$  males by mating them with  $XX$  females of the reference strains,

since  $YY$  males sire all-male broods. A  $WY$  female can be identified in still another, though indirect, way. The female parent of any  $YY$  son must have possessed the  $WY$  constitution.

*Collecting Localities.* — The following is a list of stations from which platyfish were taken for analysis of their sex chromosome constitution. The dates and the expeditions responsible for the collections are given in parentheses. The stations are listed from East to West (Text-fig. 1).

*Belize River*, British Honduras: just north of the town of Belize (1949, Gordon, Fairweather, Chaveria).

*New River*, British Honduras: 1.6 km. north of San Estevan (March, 1954, Gordon, Fairweather, Chaveria).

*Río Hondo*: (a) east bank of the east branch of Río Hondo, opposite San Antonio, British Honduras (March, 1954, Gordon, Fairweather, Chaveria).



(b) small rill on east bank of Río Hondo at Douglas, about 8 km. downstream from the previous location (March, 1963, Kallman, Rosen, Dorion, Llarena).

(c) *Aguada Corriental* at Tikal, Guatemala: (April, 1963, Kallman, Rosen). This aguada belongs to the Río Hondo drainage. Although no outlet was observed during our visit, water from this small pool overflows into a wooded, swampy depression, El Bajo de Santa Fe, in the rainy season, whence it flows through the Río Holmul into the Río Hondo.

*Lake Petén*, Guatemala: (a) near Flores at the western end of Lake Petén (1954, Gordon).

(b) small stream running into the eastern end of Lake Petén just south of Remate airstrip (April, 1963, Kallman, Rosen).

*Río Usumacinta* system: (a) Río de la Pasión — mouth of a small stream on the left bank about 4 km. below Sebol, Guatemala (March, 1963, Kallman, Rosen, Cámara).

(b) Río de la Pasión — 200 meters inside a small stream on right side of river, 8 km. below Sebol, Guatemala (March, 1963, Kallman, Rosen, Cámara).

(c) Río San Pedro de Mártir — small stream 1 km. south of airstrip at Carmelita, Guatemala (April, 1963, Kallman, Rosen).

*Río Grijalva*, Mexico: collected near Villahermosa (March, 1952, Gordon).

*Río Coatzacoalcos*, Mexico: (1948, Gordon, Atz, Wood).

*Maintenance of Fish and Recording of Data.* — As soon as they are caught, all fish are placed into plastic bags inside polystyrene containers. Several hours later the original water is discarded and replaced by clean water taken along from the collecting station. At the same time injured fish are discarded or preserved. To make sure enough breeding fish reach the laboratory, many more are collected than laboratory space permitted us to use. Fish with macromelanophore patterns are selected, since the sex chromosome constitution of such "marked" fish can more readily be determined than that of the wild type (unmarked). Sexes are not separated until their arrival at the laboratory. Then the fish from each station are counted and their pigment pattern recorded. Fish that are not completely healthy or have abrasions are placed in a 0.6% sea salts solution with a few drops of methylene blue, where they are kept until recovery.

The fish are maintained according to the method described by Gordon (1950 b). In addition, all fish less than two months old receive a feeding of live brine shrimp nauplii every

afternoon. Under these conditions, platyfish become sexually mature and are ready to mate at the age of three months. When all offspring of a particular cross are mature and exhibit their respective pigment pattern, the data are recorded in the central file of the Genetics Laboratory. The fish are preserved in alcohol or formalin and are thus available for future reference.

All wild-caught fish and their descendants and the offspring of crosses between different reference stocks are identified by a pedigree number given to them at birth or upon their arrival in the laboratory. A second number following the pedigree refers to individual fish, e.g. 1341 — 1 is female No. 1 of pedigree 1341 (the Lake Petén fish). Numbers 1-10 are reserved for females, numbers 11-20 for males. Wild-caught fish of the Belize River, New River, Río Hondo (1953 collection), Río Grijalva and Río Coatzacoalcos are identified by the symbols Bp, Np, Hp, Gp and Cp, respectively, followed by the number of the particular individual. All crosses pertaining to any one location have been listed together in one or two tables and related crosses have been grouped together wherever possible. In several cases, a male was mated with two females and all their offspring were given the same pedigree number; one of these sibships has then been called "a" and the other "b". Intrastrain crosses involving fish of the reference stocks have been summarized in Tables 2-5.

In the tables, fish belonging to any of the reference stocks have been merely identified by their code letters (Table 1). The chromosome constitution of the wild-caught fish and their descendants, as written in the tables, is the only one that will adequately explain the results of all crosses.

## RESULTS

### A. SEX DETERMINATION IN LABORATORY STOCKS OF PLATYFISH, *Xiphophorus maculatus*

*New River Strain* — Np: A single gravid female from the New River was the progenitor of this strain. Among her offspring were fish that carried the *Sd*, *N* or *Sr* alleles. All three alleles have been retained through more than 11 generations of inbreeding by mating either *N* females with *Sd Sr* males, *Sd* females with *N Sr* males or *Sr* females with *Sd N* males (Table 2). Sometimes the alleles were not expressed phenotypically, but in several instances their presence was demonstrated through additional appropriate crosses. The sex ratio of 240 females to 276 males is in good agreement with the expected 1:1 ratio. However, the possibility that at least a few of the wild type, stripe-sided, spotted-dorsal or nigra males were sex reversed (*WY* ♂) or the result of crossing over cannot



TABLE 1. LABORATORY STOCKS OF THE PLATYFISH, *Xiphophorus maculatus*\*

Geographic Origin	Year	Code	Sex Chromosome Constitution		Generations in the Laboratory
			Females	Males	
New River	1954	Np	WY	YY	16
Río Hondo	1954	Hp-1	WY	YY	15
Río Hondo	1954	Hp-2	XX	XY	18
Río Grijalva	1952	Gp	XX	XY	18
Río Coatzacoalcos	1948	Cp	XX	XY	21
Río Jamapa	1939	Jp 163 A	XX	XY	31
Río Jamapa	1939	Jp 163 B	XX	XY	26
Río Jamapa	1939	Jp 30	XX	XY	42

\* Genetics Laboratory of the New York Zoological Society located at the American Museum of Natural History, as of December, 1964.

be excluded. Only two fish among 240 female offspring inherited the pigment pattern of the female parent. Such mother-to-son inheritance is characteristic for the *WY-YY* type of sex determination. The *W* chromosome carries the wild type allele; the *N*, *Sr* and *Sd* alleles are located on the *Y* chromosomes.

One of the exceptional females died, but the other was testcrossed with a wild type *YY* (Hp-1) male.

Exceptional Np female × Hp-1 male  
 $W_N Y_{Sr} \times Y_+ Y_+$   
 F<sub>1</sub> (pedigree 1200)

Females: 44 *N*, 4+; Males: 44 *Sr*, 2 *N*

Since all but two of the nigra (*N*) offspring were females and all striped (*Sr*) offspring males, the exceptional *N Sr* female was probably the result of a crossover. The four wild type females of pedigree 1200 may have been the result of nonexpressivity of the *N* gene, which was very weakly expressed in the other females. The two nigra males were not testcrossed; they

might have been exceptional *WY* males (see similar cross in Table 10, ped. 1461 b).

*Hondo Strains* — Hp-1, Hp-2: Both strains were descended from fish collected in the Río Hondo in 1954. Hp-1, which has been inbred brother-to-sister for the last 15 generations, possesses the *WY-YY* sex-determining system. The *W* chromosome carries no macromelanophore gene; one of the *Y* chromosomes is marked by the gene *Sd* and can be traced back to the offspring of Hp-1. The *Y* chromosome carrying the wild type allele has been derived from Hp-11 (see also Table 8 for the early history of this strain). Nine generations of this strain were obtained by mating wild type females with *Sd* males. These matings resulted in four classes of offspring in approximately equal numbers:

Parents  
 $W_+ Y_+ \times Y_+ Y_{Sd}$   
 Offspring<sup>1</sup>  
 Females: 111  $W_+ Y_{Sd}$ , 115  $W_+ Y_+$ ;  
 Males: 118  $Y_+ Y_{Sd}$ , 88  $Y_+ Y_+$

<sup>1</sup> One additional *Sd* fish possessed no gonad.

TABLE 2. INHERITANCE OF PIGMENT GENES AND SEX RATIO IN THE NEW RIVER (Np) STRAIN OF *Xiphophorus maculatus*

Parents		Offspring												
Type of Cross		Females					Males							
Female	Male	<i>Sr</i>	<i>Sd</i>	<i>N</i>	<i>Sr</i>	+ <sup>1</sup>	<i>N</i>	<i>N</i>	<i>Sr</i>	<i>Sr</i> <sup>1</sup>	<i>Sd</i> <sup>1</sup>	<i>N</i> <sup>1</sup>	+ <sup>1</sup>	
$W_+ Y_N \times Y_{Sd} Y_{Sr}$		39	30	1	1	3	46	39	—	4	5	1	1	
$W_+ Y_{Sd} \times Y_N Y_{Sr}$		37	—	56	—	3	43	—	46	12	—	20	1	
$W_+ Y_{Sr} \times Y_{Sd} Y_N$		—	36	32	—	2	—	24	34	—	—	—	—	
		76	66	89	1	8	89	63	80	16	5	21	2	
		Total:					240							276

<sup>1</sup> Most of the wild-type males and females, as well as the *Sd*, *Sr* and *N* males, are due to nonpenetrance of the macromelanophore genes.

Three other generations were obtained by mating a *Sd* female with a wild type male.

$$\begin{array}{l} \text{Parents} \\ W_+ Y_{sd} \times Y_+ Y_+ \end{array}$$

Offspring

Females: 64  $W_+ Y_+$ ; Males: 61  $Y_+ Y_{sd}$

The sex ratio was 290 females to 267 males.

Strain Hp-2, which has been inbred brother-to-sister for more than 18 generations, possesses the *XX-XY* sex-determining mechanism. The *Y* chromosome is marked by the *Sd* gene. Among 614 fish raised, only two exceptional males occurred, but they were not testcrossed.

$$\begin{array}{l} \text{Parents} \\ X_+ X_+ \times X_+ Y_{sd} \end{array}$$

Offspring

Females: 305  $X_+ X_+$ ;

Males: 307  $X_+ Y_{sd}$ , 2  $X_+ ?$

*Grijalva Strain* – Gp: These platyfish, which have been inbred for the last 13 generations, possess the *XX-XY* sex-determining system. The *X* chromosomes carry either the genes *Sd* or *Sp*, and the *Y* chromosome is marked by *Sd*. The sex ratio of 373 females to 350 males (including all exceptions and individuals only partially differentiated) does not differ significantly from unity. The high incidence of only partially differentiated males may be related to pituitary abnormalities that have recently been discovered in this strain (Schreibman & Charipper, 1962).

The Gp strain can be traced back to a female (*XX*) heterozygous for the *Sp* gene and to a male ( $X_{sd} Y_+$ ). In the third inbred generation (ped. 864), an exceptional *Sp Sd* male occurred which, when mated to one of his  $X_{sd} X_{sd}$  sisters, sired offspring consisting of many *Sp Sd* females and *Sd* males and 3 exceptional *Sd* females (ped. 942, Table 3). From this series of crosses it is apparent that the exceptional male had the  $X_{sp} Y_{sd}$  constitution and had arisen as the result of a crossover.

One of the exceptional *Sd* females of pedigree 942 was testcrossed to a  $X_+ Y_{sr}$  male (a hybrid between a Hp-2 ♀ and a Np ♂).

$$\begin{array}{l} \text{Female 942} \quad \text{Male} \\ X_{sd} Y_{sd} \times X_+ Y_{sr} \end{array}$$

F<sub>1</sub> (pedigree 1010)

Females: 8 *Sd*; Males: 53 *Sd Sr*, 15 *Sd*

Although the frequencies of the three classes of offspring differed significantly from expectation, the unusual sex ratio and the inheritance of the *Sr* gene by the males only indicates that the exceptional female had the *XY* genotype.

The exceptional *Sd* male of pedigree 718 was also tested. When mated to a Jp 30 female ( $X_{sr} X_{sr}$ ), 20 *Sr* females and 18 *Sd Sr* males were produced. The exceptional male must have resulted from a crossover.

*Coatzacoalcos Strain* – Cp: These fish possess the *XX-XY* sex-determining mechanism. They have been bred by four types of brother-to-sister matings for the last 17 generations.

TABLE 3. INHERITANCE OF PIGMENT PATTERN AND SEX RATIO IN THE GRIJALVA (Gp) STRAIN OF *Xiphophorus maculatus*

Ped. No.	Parents		Offspring							
	Female	Male	Females				Males			
			<i>Sd</i>		<i>Sp</i>		<i>Sd</i>		<i>Sp</i>	
			<i>Sd</i>	<i>Sp</i>	<i>Sp</i>	+	<i>Sd</i>	<i>Sp</i>	<i>Sp</i>	+
718	$X_+ X_{sp}$	$X_{sd} Y_+$	30	1	26	1	1	17	1	30
779	$X_+ X_{sd}$	$X_{sp} Y_+$	—	16	12	—	16	—	—	14
864	$X_{sp} X_{sd}$	$X_{sd} Y_+$	14	—	7	—	15	15	1	—
942	$X_{sd} X_{sd}$	$X_{sp} Y_{sd}$	3	—	27	—	23	—	—	—
c m r a o n s y s e	$X_{sp} X_{sd}$	$X_{sd} Y_{sd}$	58	—	71	—	61 <sup>1</sup>	—	53 <sup>1</sup>	—
			—	—	84 <sup>2</sup>	—	87 <sup>3</sup>	—	—	—
			—	13	10	—	12 <sup>1</sup>	—	4	—

Total Number – Females: 373; – Males: 350; – No Gonads: 1

<sup>1</sup> Fourteen of these 126 males possessed a modified anal fin that was arrested in its development, although the fish were five to six months old.

<sup>2</sup> Several females had undeveloped gonads when sacrificed.

<sup>3</sup> One additional fish had a well differentiated gonopodium, but no testis could be found upon autopsy.

TABLE 4. INHERITANCE OF PIGMENT PATTERN AND SEX RATIO IN THE COATZACOALCOS (Cp) STRAIN OF *Xiphophorus maculatus*<sup>1</sup>

Parents		Offspring					
Female	Male	Females		Males			No Gonad
		<i>Sp</i>	+	<i>Sd</i>			
		<i>Sp</i>	+	<i>Sp</i>	<i>Sd</i>	+	<i>Sp</i>
<i>X<sub>Sp</sub>X<sub>Sp</sub></i>	<i>X<sub>+</sub>Y<sub>Sd</sub></i>	15	—	17	—	—	—
<i>X<sub>Sp</sub>X<sub>+</sub></i>	<i>X<sub>+</sub>Y<sub>Sd</sub></i>	73 <sup>2</sup>	77 <sup>2</sup>	84	80	1	—
<i>X<sub>Sp</sub>X<sub>+</sub></i>	<i>X<sub>Sp</sub>Y<sub>Sd</sub></i>	16	—	12	8	—	—
<i>X<sub>+</sub>X<sub>+</sub></i>	<i>X<sub>Sp</sub>Y<sub>Sd</sub></i>	20	—	—	21	—	6 <sup>3</sup>
Total: 201 ♀ ; 223 ♂ ; 6 no gonad.							

<sup>1</sup> For 12 out of 17 generations.  
<sup>2</sup> Many females isolated in an aquarium died and were not recorded.  
<sup>3</sup> These fish occurred among the offspring of a single female.

Complete records are available for twelve generations (Table 4). The excess of males is due to the accidental loss of an aquarium of females, the number of which has not been recorded. The six *Sp* fish with no gonad occurred among the offspring of a single female. The single exceptional wild type male was not test-crossed.

*Jamapa Strains* — Jp 30, Jp 163 A, Jp 163 B: The origin of strains 30 and 163 has been described by Gordon (1947) who showed that Jamapa fish possess the *XX-XY* sex-determining

mechanism. Strain 30 is now in its 42nd generation of inbreeding. Both its *X* and *Y* chromosomes are marked by gene *Sr*. The sex ratio has not always been recorded, but complete data are available for the 35th and the 38th to 42nd generations inclusive (Table 5).

During the first six generations of inbreeding, the *X* chromosomes of strain 163 were marked either by gene *Sd* or *Sp*. Subsequently, strain 163 was split into two sublimes, A and B. In the A line, which is now in its 30th generation of inbreeding, the *Sd* gene is located on the

TABLE 5. SEX RATIO IN JAMAPA (Jp) STRAINS OF *Xiphophorus maculatus*

Parents		Offspring								
Female	Male	Females					Males			
		<i>Sd</i>	<i>Sp</i>	<i>Sr</i>	<i>Sd</i>	<i>Sp</i>	<i>Sr</i>	<i>Sd</i>	<i>Sp</i>	<i>Sr</i>
<i>Strain Jp 30</i>										
<i>X<sub>Sr</sub>X<sub>Sr</sub></i>	<i>X<sub>Sr</sub>Y<sub>Sr</sub></i>	—	—	137	—	—	—	—	141	—
<i>Strain Jp 163 B</i>										
<i>X<sub>Sp</sub>X<sub>Sp</sub></i>	<i>X<sub>Sp</sub>Y<sub>Sr</sub></i>	—	387	—	—	9	—	—	—	386
<i>Strain Jp 163 A</i>										
<i>X<sub>Sd</sub>X<sub>Sd</sub></i>	<i>X<sub>Sd</sub>Y<sub>Sr</sub></i>	369	—	—	31	—	2	—	—	310

DISTRIBUTION OF EXCEPTIONAL *XY* FEMALES IN STRAIN Jp 163 A

Generation	Mating	Offspring		
		<i>X<sub>Sd</sub>X<sub>Sd</sub></i> ♀	<i>X<sub>Sd</sub>Y<sub>Sr</sub></i> ♀	<i>X<sub>Sd</sub>Y<sub>Sr</sub></i> ♂
14	a	5	8	2
17	a	18	1	10
17	b	17	5	16
24	a, b	27	14	16
24	c	1	1	2
28	a, b, d	44	0	17
28	c	41	1	21
31	a	15	1	12
31	b	17	0	10



X chromosome, while in the B line, which has been inbred for 26 generations, the X chromosome is marked by *Sp*. In both strains, the Y chromosome is marked by gene *Sr*. In the past the sex ratio of both strains has only been recorded every second or third generation unless "exceptional" fish occurred. Therefore, the percentage of exceptions in both strains is actually much lower than appears from Table 5. Both strains are characterized by the sporadic occurrence of XY females (MacIntyre, 1961). In strain 163 B, all nine exceptional females were found among the offspring of a single fish of the 11th generation. The 31 exceptional females in strain 163 A were produced by eight females, four of which accounted for 27 of the exceptions (Table 5). The two exceptional *Sd* males of strain 163 A resulted from crossovers between the X and Y chromosomes. When these males were mated to females of strain 163 B, 50 of the offspring were *Sp Sd* females and 55 *Sp Sd* males (ped. 1297, 1700). The exceptional males must have been homozygous for the *Sd* gene.

B. THE SEX CHROMOSOME MECHANISM OF WILD POPULATIONS OF *Xiphophorus maculatus*

Belize River

In 1950 Gordon briefly mentioned that platyfish collected in the Belize River possessed the *WY-YY* sex-determining mechanism. However, no detailed experiments were ever published. The crosses on which Gordon based his conclusion are therefore listed here (Table 6).

Two wild-caught males were mated with Jamapa (*XX*) females. From these crosses 304 offspring were obtained, all males.<sup>2</sup> This is conclusive evidence that the males had the *YY* constitution.

Two wild-caught females (Bp-1, Bp-7) and the daughters of two others (Bp 3<sup>2</sup>, Bp 9<sup>2</sup>) were mated with their own Belize males or with known *XY* males. The paternal pigment patterns were inherited by one half of the male and female offspring (ped. Bp-1<sup>2</sup>, 309, 308) while the pigment pattern of the female parent was inherited only by the sons (ped. Bp-1<sup>2</sup>, 329). This type of inheritance is diagnostic for the *WY-YY* system. However, these crosses do not rule out the possibility that one of the progenitors may also have possessed an X chromosome.

New River

All knowledge about the sex-determining

<sup>2</sup> Gordon (1951 a) listed the offspring of one of these crosses as 239 males and one female. But in the files of the Genetics Laboratory the entry under this pedigree lists only 239 males.

TABLE 6. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF WILD-CAUGHT *Xiphophorus maculatus* FROM THE BELIZE RIVER

Ped. No.	Parents		Offspring											
	Female	Male	Females					Males						
			<i>Sp</i>	<i>Sd</i>	<i>Sr</i>	<i>N</i>	+	<i>Sr</i>	<i>Sp</i>	<i>Sd</i>	<i>N</i>	<i>N</i>	<i>Sd</i>	<i>Sr</i>
304	$X_{Sr} X_{Sd}$	$Y + Y_{Sd}$	—	—	none	—	—	34	—	—	—	—	—	—
305	$X_{Sp} X_{Sd}$	$Y + Y +$	—	—	none	—	—	—	123	114	—	—	31	—
Bp-1 <sup>2</sup>	$W + Y_{Sd}$	$Y_N Y +$	—	—	—	21	12	—	—	7	—	—	—	2 <sup>1</sup>
309*	$W + Y_{Sd}$	$Y_N Y_{Sd}$	—	14	—	15	2	—	—	14	5	4	—	2
329	$W + Y_{Sd}$	$X_{Sr} Y_{Sr}$	—	—	26	—	—	—	—	—	—	—	26	—
308	$W + Y +$	$X_{Sp} Y +$	19	—	—	—	27	—	22	—	—	—	—	36

<sup>1</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.

\* The macromelanophore genes of the Belize River population vary greatly in their expression; in some individuals they do not manifest themselves.

TABLE 7. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE DESCENDANTS OF WILD-CAUGHT *Xiphophorus maculatus* FROM THE NEW RIVER

Ped. No.	Parents				Offspring									
	Female		Male		Females				Males					
					<i>Sd</i>	<i>N</i>	+	<i>Sr</i>	<i>Sd</i>	<i>Sp</i>	<i>N</i>	<i>Sd</i>	<i>Sr</i>	+
557	Cp	$X_{Sp} X_+$	Np-11	$Y_+ Y_+$	—	none	—	—	—	19	—	—	—	18
529	Jp	$X_{Sp} X_{Sd}$	Np-12	$Y_+ Y_+$	—	none	—	—	—	72	—	—	—	75 <sup>1</sup>
530 <sup>2</sup>	Np-1	$W_+ Y_{Sr}$	unknown		9	1	13	6	3	—	2	—	7	6
563 <sup>3</sup>	530-1	$W_+ Y_{Sd}$	530-13	$Y_{Sr} Y_N$	—	24	19	—	24	—	—	16	—	—
564 <sup>3</sup>	530-3	$W_+ Y_N$	530-11	$Y_{Sr} Y_{Sd}$	5	—	6	—	—	—	8	4	—	—
649 <sup>3</sup>	563-1	$W_+ Y_N$	530-14	$Y_{Sd} Y_{Sr}$	16	—	22	—	1	—	19	8	—	—

<sup>1</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.  
<sup>2</sup> Some fish were sacrificed before pigment patterns developed fully.  
<sup>3</sup> Fish not scored for *Sr* pattern.

mechanism of the platyfish population of the New River has been derived from a single female and two males (Table 7). Both males possessed the *YY* constitution, since crossing them to Jamapa or Coatzacoalcos *XX* females resulted in all-male broods. The female that appeared to have been fertilized by several males in nature, had the *WY* constitution. When one of her *Sd* daughters was mated with one of her *N* sons, one half of the male and female offspring inherited the *N* gene, while *Sd* was inherited only by males. The same type of inheritance was observed when a female (*N*) was mated to a male (*Sd Sr*). Inbreeding this pedigree (649) gave rise to the Np strain. The exceptional *Sd* male of pedigree 649 was not testcrossed. It could have been a *WY* male (sex reversal), a crossover between the *W* and *Y* chromosome, or the result of nonexpressivity of the *N* gene.

Río Hondo Drainage

Three collections from this river system were available for analysis. Two were made in the Río Hondo at San Antonio (1954) and Douglas (1963) and one in an isolated aguada in the extreme headwater region at Tikal (1963).

*Río Hondo (San Antonio, 1954)*: The crosses pertaining to this collection have been summarized in Table 8. Male Hp-11, when crossed with a Jamapa *XX* female, gave rise to offspring of both sexes (ped. 520), indicating that he possessed the *XY* constitution. Hp-1 was a *WY* female; when one of her *Sd* sons was mated to Hp-10, all-male broods were produced (ped. 552). This cross also demonstrates that female Hp-10 was *XX*. Hp-2 must have been a *WY* female. When one of her sons was testcrossed with a Jamapa *XX* female, he sired all-male offspring (ped. 558). Female Hp-3 gave rise to

TABLE 8. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE DESCENDANTS OF WILD-CAUGHT *Xiphophorus maculatus* FROM THE RÍO HONDO (SAN ANTONIO, 1954)

Ped. No.	Parents				Offspring								
	Female		Male		Females				Males				
					<i>Sp</i>	<i>Sd</i>	+	<i>Sd</i>	<i>Sr</i>	<i>Sd</i>	<i>Sp</i>	<i>Sr</i>	+
520	Jp	$X_{Sp} X_{Sd}$	Hp-11	$X_+ Y_+$	5	2	—	—	—	—	4	—	7
527a	Hp-1	$W_+ Y_+$	unknown		—	1	2	4	—	—	—	3	—
552	Hp-10	$X_+ X_+$	527a-11	$Y_+ Y_{Sd}$	—	none	—	30	—	—	—	—	12
537	Hp-2	$W_+ Y_+$	unknown		—	—	1	2	—	—	—	—	3
558	Jp	$X_{Sr} X_{Sr}$	537-11	$Y_+ Y_{Sd}$	—	none	—	—	25	34	—	—	—
Hp-3 <sup>2</sup>	Hp-3	$X_+ X_+$	unknown		—	—	6	19	—	—	—	—	6
697	Hp-3 <sup>2</sup>	$X_+ X_+$	Hp-3 <sup>2</sup>	$X_+ Y_{Sd}$	—	—	19	21	—	—	—	—	—
551	Hp-5	$W_+ Y_{Sd}$	Hp-11	$X_+ Y_+$	—	—	29	15	—	—	—	—	—
650	551-1	$W_+ ?$	551-11	$Y_+ Y_{Sd}$	—	7	7	9	—	—	—	—	—
736	650-3	$W_+ Y_+$	552-11	$X_+ Y_{Sd}$	—	16	10	16	—	—	—	—	18

a high percentage of males (ped. Hp-3<sup>2</sup>), suggesting that she was a *XX* female that had been inseminated by both *XY* and *YY* males. A mating of one of her daughters with a son (*Sd*) produced only wild type females and *Sd* males (ped. 697). This father-to-son inheritance is diagnostic for the *XX-XY* type of sex-determination. Inbreeding pedigree 697 resulted in the Hp-2 strain of *X. maculatus*. Female Hp-5, which had been kept isolated from males for eight months, was finally mated to Hp-11 (*XY*, see ped. 520). Since this cross produced only wild type females and *Sd* males, Hp-5 must have been a *WY* female, with the *Sd* gene on the *Y* chromosome. Hp-5 is a progenitor of the Hp-1 line; its *W* chromosome can be traced back to this female. Her offspring (ped. 551) were inbred (ped. 650). A wild type *WY* female of this brood was mated with an  $X_+ Y_{sa}$  male of pedigree 552. Inbreeding of their offspring (ped. 736) resulted in the Hp-1 line. The *X* chromosome was eliminated three generations later when a *WY* female was mated to a  $Y_{sa} Y_+$  male.

*Río Hondo (Douglas, 1963)*: Four wild-caught males from Douglas (ped. 1335) were testcrossed with *XX* females belonging to the Jamapa and Grijalva reference stocks. One male gave rise to males and females in approximately equal numbers, indicating that he possessed the *XY* genotype. The other three males must have been *YY* fish, since in all the crosses only male offspring were produced (Table 9).

Eight females from the Douglas location (ped. 1335) possessed the *WY* chromosome constitution. A ninth female was apparently an exceptional *WW* fish (Table 10). Seven sons of five of the *WY* females were testcrossed with *XX* females of the Jamapa and Coatzacoalcos stocks; they gave rise to 338 offspring, all males (ped. 1506, 1482 a, 1461 a, 1486, 1480, 1479,

1475). But when two of the males were mated to known *WY* females of the New River reference stock, the offspring consisted of both sexes in about equal frequencies (ped. 1482 b, 1461 b). Further evidence that these wild-caught Douglas females were *WY* was provided by crossing seven of their daughters with *XY* males of the Jamapa and Grijalva strains. One-half of the female offspring exhibited the *Y*-linked pigment pattern of the male parent, and one-half of the male offspring inherited the pigment gene located on the *X* chromosome of the father. The female parents must, therefore, have possessed the *WY* genotype (ped. 1459, 1467, 1508 a and b, 1540, 1557, 1460). In addition, the pigment pattern of four wild-caught females (1335-1, -2, -3, -4) was inherited only by their sons, again strong evidence for the *WY* genotype. Two females, 1335-7 and -9, were shown to be *WY* by testcrossing them directly with *XY* Jamapa males (ped. 1429, 1520). Similarly, female 1335-8 was *WY*. Although she produced only three young, her *WY* genotype was established by testcrossing each of them. One daughter possessed the *WY* genotype, the other was a *WX* female, and the only son was a *XY* male (ped. 1566, 1588, 1555).

Female 1335-6 appears to have been *WW*. When she was mated to a  $X_{sp} Y_{sr}$  Jamapa male, the offspring consisted of equal numbers of *Sp* and *Sr* females, but no males (ped. 1422). Although only 29 fish were reared, it is unreasonable to attribute the absence of males to a recessive lethal gene. Even if such a gene had existed on the "*Y*" chromosome of the female parent and also on the *Y* chromosome of the Jamapa strain, at least one type of male (the *XY* class) should have been found among the offspring. It is also possible that 1335-6 had the *WX* genotype, however, since the *X* chromosome is present in the Río Hondo platyfish popu-

TABLE 9. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF FOUR WILD-CAUGHT MALE *Xiphophorus maculatus* FROM THE RÍO HONDO (DOUGLAS, 1963)

Parents					Offspring				
Female			Male		Females		Males		
Ped. No.	Reference Strain		Wild-caught						
1356a	Jp	$X_{sp} X_{sp}$	1335-11	$X_+ Y_+$	<i>Sp</i>	<i>Sd</i>	<i>Sp</i>	<i>Sd</i>	+
1356b	Gp	$X_{sd} X_{sd}$	1335-11	$X_+ Y_+$	27	—	27	—	—
1371a	Jp	$X_{sp} X_{sp}$	1335-14	$Y_+ Y_+$	9	15	10	4	—
1371b	Gp	$X_{sd} X_{sd}$	1335-14	$Y_+ Y_+$	none	—	40	—	—
1385	Jp	$X_{sp} X_{sp}$	1335-15	$Y_+ Y_+$	none	—	19	19	—
1387a	Jp	$X_{sp} X_{sp}$	1335-16	$Y_+ Y_+$	none	—	66	—	—
1387a	Jp	$X_{sp} X_{sp}$	1335-16	$Y_+ Y_+$	none	—	28	—	—
1387b	Gp	$X_{sd} X_{sd}$	1335-16	$Y_+ Y_+$	none	—	6	8	—



lation. In this case the sex ratio would have been  $2 Sp \text{ } \varphi : 1 Sr \text{ } \varphi : 1 Sr \text{ } \delta$ . Again, the probability that no males would occur among 29 offspring is extremely small. Moreover, the observed ratio of *Sp* and *Sr* females is not in accordance with the expectation ( $\chi^2 = 14$ ;  $n = 2$ ;  $p < .01$ ). On the other hand, if the Douglas female had the *WW* genotype, the offspring should consist of equal numbers of *Sp* and *Sr* females and no males, and the actual result fits this theoretical expectation very well. The *WW* female probably arose from a mating between a *WY* female and an exceptional *WY* male. Such males have been reported to occur sporadically in laboratory stocks (Bellamy & Queal, 1951; Breider, 1942; Gordon, 1951 a; Öktay, 1959 a, b) and are also to be expected in nature. A male offspring of a wild-caught female from another location of the Río Hondo drainage possessed the *WY* genotype (Table 12, ped. 1363 b).

Other exceptional fish were observed among the descendants of the Douglas platyfish. One of the wild type males of pedigree 1461 b must have had the *WY* genotype, since males and females were produced when it was mated to a *XX* female (ped. 1668). When mated to a  $W_+ Y_{sd}$  female of the Hp-1 reference stock, the same male sired a brood that consisted of 90% females, half of them exhibiting the *Sd* pattern of the female parent (ped. 1636). These results are in agreement with the assumption that the male had the *WY* genotype. The other exceptional male of pedigree 1461 b was not tested. The wild type and *Sd* males of pedigree 1482 b resulted from nonexpression of the *N* gene (ped. 1689, 1737).

The two exceptional *Sd* males and females of pedigree 1555 presumably had the *Sp Sd* genotype. In this pedigree the *Sp* pattern was sometimes weakly developed while the *Sd* gene was strongly expressed, often accompanied by a "spillover" from the dorsal fin onto the flanks. Therefore, the *Sp* is sometimes masked by the *Sd*. The exceptional females died before being testcrossed. They could have been either *WY* females (the result of a crossover) or *XY* females (sex reversal).

*Aguada Corriental (Tikal)*: Six wild-caught males (ped. 1343) from this small pool were tested directly with *XX* females of three reference stocks (Gp, Jp 163 A and B), four of them with two females each. Since the crosses resulted in 374 offspring, all of them males (Table 11), the wild-caught fish must have possessed the *YY* chromosome constitution.

Evidence from three types of matings shows that all Tikal females (ped. 1343) were of the

*WY* genotype (Table 12). A total of 449 young were obtained, all males, when eleven of the sons of wild-caught fish were mated with *XX* Jamapa females, (ped. 1529, 1440, 1495, 1556, 1523, 1533, 1505, 1660, 1565, 1625, 1441). When two of the sons were mated to the daughters of wild-caught Tikal females, males and females were produced in a 1:1 ratio (ped. 1468, 1435). In addition, some of the daughters of wild-caught females were mated to *XY* males of reference strains. In each cross, one-half of the female offspring inherited the pigment gene located on the *Y* chromosome of the male parent (ped. 1438, 1442, 1538 a and b, 1539, 1534 a and b, 1535, 1547). The results of all of these crosses could only be consistent with the assumption that Tikal females possess the *WY* genotype.

The Tikal matings gave rise to a single exception — the wild type male found among the offspring of female 1343-4 (ped. 1363 b). All the sons should have exhibited the *Nigra* gene which she carried on the *Y* chromosome. In order to determine whether the exceptional male was *WY* or a *YY* male that arose from a crossover between the *W* and *Y* chromosome, it was mated to a Jamapa (*XX*) female. The mating resulted in males and females in approximately equal numbers (ped. 1531), and this is strong evidence that the genotype of the exceptional fish was *WY*. That the females of pedigree 1531 had indeed inherited a *W* chromosome from their father was demonstrated by crossing one with a Jamapa (*XY*) male (ped. 1609). This cross resulted in a sex ratio of 3 females to one male, and about one-third of the females exhibited the *Y*-linked *Sr* trait of the Jamapa male.

#### Lake Petén

Two platyfish collections were made in Lake Petén, one near Flores at the western end (1954) and the other near Remate at the lake's easternmost tip (1963).

*Lake Petén (1954)*: The crosses pertaining to this collection have been summarized in Table 13. Three males, when mated to *XX* females, produced both male and female offspring and must have possessed the *XY* genotype (ped. 532, 550, 549). A fourth male was apparently *YY* (ped. 545). The sex chromosome constitution of only a single wild-caught female, Pp-1, was identified. The analysis is quite complicated, since only one macromelanophore pattern, Spotted-dorsal (*Sd*), was present among her offspring and this showed great variation in expressivity, and in several cases no penetrance at all. Pedigrees 626, 640, 574 and 602 indicate that Pp-1 possessed a *W* chromosome. Two male descendants, 574-13



TABLE 10. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF NINE WILD-CAUGHT FEMALE *Xiphophorus maculatus* FROM THE RÍO HONDO (DOUGLAS, 1963) (Continued)

Ped. No.	Parents		Offspring														
	Females	Male	Females						Males								
			Sp	Sd	Sr	N	+	Sp	N	Sd	Sr	N	Sd	Sr	N	+	
1588	1488-2	W <sup>+</sup> X <sub>Sp</sub>	Cp	X <sup>+</sup> Y <sub>Sd</sub>													
1555	1488-1	W <sup>+</sup> Y <sub>Sd</sub>	JP	X <sub>Sp</sub> Y <sub>Sr</sub>													
1520	1335-9	W <sup>+</sup> Y <sup>+</sup>	JP	X <sub>Sp</sub> Y <sub>Sr</sub>													

<sup>1</sup> Nonpenetrance of N gene. Phenotype of 1482 b-11 was Sd; 1482 b-12 was wild type.  
<sup>2</sup> Nonpenetrance of Jamapa Sd in inter-river hybrids.  
<sup>3</sup> Presumably SdSp, but strong expressivity of Sd masks the weak Sp.

and 602-11, sired all-male broods and must have had the YY constitution (ped. 698 and 781). Consequently, two daughters of Pp-1 (533-3 and -5) were WY females. Unless one assumes that the wild type phenotype of the 15 males of pedigree 595 resulted from nonpenetrance of the Sd gene, there is good evidence that a third daughter (533-8) was XX. The sex ratio of pedigree 654 also shows that one son, 533-19, was XY. All crosses indicate that Pp-1 was a WX female and was fertilized by at least two males, one XY the other YY. The son of a second Petén female, Pp-2, appears to have been XY (ped. 595).

*Lake Petén* (1963): The crosses involving this platyfish collection (ped. 1341) also indicate that both W and X chromosomes are present. Seven wild-caught males were tested with XX females of the Jamapa and Grijalva reference stocks (Table 14); four were found to possess the XY genotype (ped. 1396 a and b, 1437, 1408, 1413) and three the YY genotype (ped. 1388 a and b, 1416, 1439).

Among the offspring of one Petén male (1341-16), six exceptional fish occurred (ped. 1413). The other males of this pedigree exhibited the expected Sp Sd phenotype. Since the Sd pattern was strongly developed, it seemed unlikely that the Sp males had resulted from nonpenetrance of the Sd gene. When two of these exceptional males were testcrossed with XX-Jamapa females, all-female broods resulted (ped. 1580, 1595), clearly indicating that they were XX. The cause of the high incidence of sex-reversed males (XX) is not apparent.

Of seven wild-caught Petén females tested (Table 15), one possessed the WX, another the XX and four the WY genotype. For one female our analysis is incomplete; only a W chromosome was identified. Female 1341-1 exhibited the Nigra pattern and must have possessed the WX genotype. One of her wild type daughters was WY; when she was mated with a X<sub>Sp</sub> Y<sub>Sr</sub> Jamapa male, the Sp and Sr patterns were inherited by both sexes (ped. 1522). Her Nigra daughter, however, was XX; when she was mated to a Jamapa male, the Sr gene was inherited by the male offspring only (ped. 1562). The sons of female 1341-1 were XY males (ped. 1485, 1490).

Among the descendants of this Petén female an unusually large number of males and females appeared that exhibited the pigment pattern of the opposite sex. In pedigree 1562, all males should have been Sr, but one exhibited only the N pattern. It was mated to a Jamapa (XX) female. Among 57 offspring, ten were males (ped. 1685). Because of the small per-



TABLE 11. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF SIX WILD-CAUGHT MALE *Xiphophorus maculatus* FROM THE AGUADA CORRIENTAL (TIKAL)

Ped. No.	Parents				Offspring					
	Female		Male		Females	Males				
	(Reference Strain)		(Wild-caught)			<i>Sp</i>	<i>Sd</i>	<i>Sr</i>	<i>Sr</i>	+
1364a	Jp	$X_{Sp} X_{Sp}$	1343-13	$Y^+ Y^+$	none	60	—	—	—	—
1364b	Gp	$X_{Sd} X_{Sd}$	1343-13	$Y^+ Y^+$	none	—	54	—	—	—
1367a	Jp	$X_{Sp} X_{Sp}$	1343-14	$Y^+ Y^+$	none	30	—	—	—	—
1367b	Gp	$X_{Sd} X_{Sd}$	1343-14	$Y^+ Y^+$	none	—	34	—	—	—
1393a	Jp	$X_{Sp} X_{Sp}$	1343-15	$Y^+ Y^+$	none	35	—	—	—	—
1393b	Gp	$X_{Sp} X_{Sd}$	1343-15	$Y^+ Y^+$	none	13	14	—	—	—
1407a	Jp	$X_{Sp} X_{Sp}$	1343-16	$Y_{Sr} Y^+$	none	19	—	19	—	—
1407b	Gp	$X_{Sp} X_{Sd}$	1343-16	$Y_{Sr} Y^+$	none	10	8	7	5	—
1414	Jp	$X_{Sd} X_{Sd}$	1343-17	$Y^+ Y^+$	none	—	—	—	—	39 <sup>1</sup>
1427	Jp	$X_{Sd} X_{Sd}$	1343-18	$Y^+ Y^+$	none	—	—	—	—	27 <sup>1</sup>
Total Number: 374										

<sup>1</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.

centage of male offspring (18%) and the inheritance of the *Nigra* pattern by both sexes, it is concluded that the exceptional male of pedigree 1562 had the *XX* genotype. If this is so, then the ten males of ped. 1685 should also have had two *X* chromosomes, but none of these was ever testcrossed.

In pedigree 1485, all females should have exhibited the *Nigra* and males the *Spot-sided* pattern. However, one exceptional *Sp* female was discovered. In a mating with a Jamapa male, she behaved like a typical *XX* female (ped. 1649). This female, therefore, arose as a result of crossing over between the *X* and *Y* chromosomes. Among her 143 offspring, four exceptional fish, two males and two females, were detected. In view of the rarity of crossing over between the *X* and *Y* chromosomes in Jamapa strains (Tables 5, 24), the two females presumably had the *XY* and the males the *XX* genotype.

In pedigree 1490, a cross very similar to the one just described, the *Nigra* pattern should have been inherited only by the females, but a single *N* male occurred. This male did not arise as a result of crossing over; in a mating with a Jamapa *XX* female he sired 71 females and two males (ped. 1570). The *Nigra* male and its two male offspring therefore possessed the *XX* constitution. Both males of pedigree 1570 were backcrossed once more to the Jamapa strain. One male gave rise to 53 offspring, all females, the other to 71 females and ten males (ped. 1686, 1687).

The genotype of female 1341-2 was identified

as *XX*. One of her *Nigra* and two of her wild type sons proved to be *XY* males (ped. 1550, 1576, 1590). One of her wild type daughters, when mated to an unrelated Petén male, was shown to be a *XX* female (ped. 1498 a; listed among the offspring of 1341-4).

Petén females 1341-3 and -4 had the *WY* chromosome constitution; some of their sons when mated to *XX* Jamapa females sired all male broods (ped. 1574, 1579). Other sons proved to be *XY* males (ped. 1504, 1498 a and b, 1496 b). Some of the daughters of the Petén females possessed the *WY*, others the *WX* constitutions. Both classes of females were mated with *XY* males, the *X* and *Y* chromosomes marked by different pigment genes. The *WX* genotype of some daughters was demonstrated by a 3:1 sex ratio and the fact that the female offspring consisted of three pigment classes and the males of only one (ped. 1575 a and b, 1513 a and b). The *WY* genotype was identified by a 1:1 sex ratio and the inheritance of the *Y*-linked pigment pattern of the father by both sexes (ped. 1524, 1496 a). The results also indicate that both wild-caught Petén females had been fertilized by *XY* males.

Among the descendants of these females, six fish exhibited the pigment patterns of the opposite sex (two females of pedigree 1504, one female of pedigree 1657, one female and one male of pedigree 1498 a, one male of pedigree 1496 b). Four proved to be crossovers (ped. 1657, 1656, 1618, 1670). The high percentage of crossing over in these sibships is quite un-

usual, in view of the low incidence of crossing over in other crosses. In pedigree 1498 a, among 62 fish, there were at least two crossovers. The exceptional *Sr* female of pedigree 1657 was a *XY* female (ped. 1724). One wild type female of pedigree 1504 was not tested.

Petén females 1341-8 and -9 were kept isolated in the laboratory for six months (no broods produced) and then mated with Jamapa males. From the inheritance of the pigment patterns among the offspring, it is obvious that they were both *WY* females (ped. 1447, 1451). The analysis of the sex chromosome constitution of Petén female 1341-5 is incomplete. Her two sons died before they could be tested. Since three of her daughters possessed the *WY* genotype (ped. 1516, 1559 a and b), this wild-caught Petén female must have possessed a *W* chromosome.

#### Río Usumacinta System

Three collections of platyfish were made in the Río Usumacinta system, two near the origin of the Río de la Pasión at Sebol and one in the headwaters of the Río San Pedro.

*Río de la Pasión:* The two collections of platyfish from the Río de la Pasión were given pedigree No. 1327 and 1328. These fish or their descendants will also be referred to as the "Sebol" platyfish.

Five wild-caught males were tested directly by mating them with one or two *XX* females of the *Jp*, *Gp* and *Hp-2* reference stocks (Table 16). Since the sex ratio of the offspring was of primary importance, some were sacrificed before their pigment pattern had developed. The five males were found to possess the *XY* constitution. The sex ratio of only one cross (ped. 1362 a) differed significantly from the expected 1:1 ratio ( $\chi^2 = 8.9$ ,  $p < .01$ ). In order to determine whether the paucity of males was somehow related to the *Y* chromosome of male 1327-14, one of his sons (ped. 1362 a) was crossed with a Jamapa *XX* female. This cross resulted in 50 females and 56 males, a good 1:1 ratio. Similarly, when male 1327-14 was mated to a second *XX* female belonging to the Grijalva strain, a normal sex ratio was obtained (ped. 1362 b). The exceptional *Sp Sd* male of pedigree 1347 b was not testcrossed.

Six Sebol females were tested; four exhibited the *WY* and one the *XX* genotype. One female, for which the analysis is incomplete, possessed at least one *X* chromosome (Table 17).

A male offspring of this Sebol female (1327-1) was *XY* (ped. 1476). One daughter was *XX*, since only male offspring were obtained when she was mated to a *YY* Sebol male (ped. 1419).

Since no other daughters of female 1327-1 were tested, it cannot be decided whether she was *WX* or *XX*. All evidence indicates that female 1328-2 possessed two *X* chromosomes. The sex ratio and the pigment patterns of her offspring (ped. 1336) strongly suggest that she was fertilized by two *XY* males, one *X<sub>sd</sub>* and the other wild type. Four of her daughters, three *Sd* and one wild type, were tested in three different ways; all proved to be *XX* (ped. 1424, 1507 a and b, 1510). The single son tested proved to be *XY* (ped. 1466).

The sex chromosome constitution of Sebol females 1327-3, 1328-1, -3 and -4 was identified as *WY*, since each of them gave rise to sons that produced all-male offspring when mated to *XX* females of the reference strains or from the Sebol location (ped. 1477, 1469 b, 1537, 1456 b, 1478 a). But when the same males were mated to their sisters or to other Sebol females carrying a *W* chromosome, male and female offspring were produced in nearly equal numbers (ped. 1469 a, 1456 a, 1478 b). The existence of a *W* chromosome among the daughters of three of the wild-caught females was also demonstrated by mating their daughters to *XY* males (ped. 1420, 1421, 1517 a and b, 1525).

It was also shown that Sebol male 1327-12, which was not tested with any of the reference stocks, must have possessed the constitution *X<sub>sp</sub> Y<sub>+</sub>* (ped. 1420). The 3:1 sex ratio and the fact that the pigment pattern of the male parent was exhibited only by some females while all sons were wild type, is in agreement with the assumption that female 1352-1 was *WX* and male 1327-12 was *X<sub>sp</sub> Y<sub>+</sub>*. Since the mother of 1352-1 was a *WY* female, she must have been fertilized in nature by a *XY* male. The existence of *WX* and *XX* fish among the female offspring of pedigree 1420 was verified through additional crosses (ped. 1511 a and b, 1512).

Similarly, male 1328-16 that fertilized female 1328-4 must have possessed the *XY* genotype, since two of four daughters tested proved to be *WX* females. When crossed with *XY* Jamapa males, they gave rise to offspring with a 3:1 sex ratio, and the *X* chromosome of the male was inherited only by the females (ped. 1517 b, 1525).

Among the progeny of the crosses listed in Table 17, only a single exceptional fish was discovered: the *Sp* female of pedigree 1421. This fish was mated with an Jamapa male (*XY*) homozygous for the *Sr* gene. Since the *Sp* gene showed strictly maternal inheritance (ped. 1610), it is concluded that the exceptional female resulted from a crossover between the *W* and *Y* chromosomes.





TABLE 12. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF EIGHT WILD-CAUGHT FEMALE *Xiphophorus maculatus* FROM THE AGUADA CORRIENTAL (TIKAL) (Continued)

Ped. No.	Parents		Male	Offspring									
	Female			Females			Males						
				<i>Sd</i>	<i>Sp</i>	<i>Sr</i>	<i>N</i>	<i>Sd</i>	<i>Sr</i>	<i>N</i>	<i>Sp</i>	<i>Sd</i>	
1625	Jp	$X_{sa} X_{sa}$	1448-12	—	—	—	+	—	—	—	—	—	+
1357	1343-8	$W_+ Y_+$	unknown	—	—	—	—	—	—	—	—	—	24 <sup>1</sup>
1441	Jp	$X_{sp} X_{sp}$	1357-11	—	—	—	3	—	—	—	—	—	1
1547	1357-1	$W_+ Y_+$	Jp	—	22	27	—	—	—	—	61	24	—
				—	—	—	—	—	—	—	18	—	—

<sup>1</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.<sup>2</sup> Some fish presumably *N Sr*; fish were sacrificed when three months old before *N* pattern had developed.

Río San Pedro: Thirteen platyfish (ped. 1342) were collected at Carmelita after several hours of seining. Five differentiated into males, seven into females and one died. None exhibited any macromelanophore pigment patterns. All the crosses involving these fish and their descendants have been summarized in Table 18.

One male, when mated to Jamapa and Grijalva *XX* females, gave rise to male and female offspring in approximately equal frequencies and, therefore, must have had the *XY* genotype (ped. 1395 a and b). Four males, when tested with *XX* reference stocks, sired all-male broods, indicating that they possessed two *Y* chromosomes (ped. 1399 a and b, 1455, 1445, 1472). In contrast, when three of these *YY* males were mated with five Carmelita females, both male and female offspring were obtained (ped. 1405 a and b, 1412, 1425 a and b). The sex chromosomes of the five wild-caught females were identified by mating a male  $F_1$  of each intra-Carmelita cross with Jamapa *XX* females. Only male offspring were obtained from all the crosses (ped. 1521, 1514, 1518, 1528, 1526). The  $F_1$  males must have possessed the *YY* constitution and the five wild-caught females must have been *WY*. In two cases, the existence of a *W* chromosome was also demonstrated by mating a female  $F_1$  of an intra-Carmelita cross with a Jamapa  $X_{sp} Y_{sr}$  male (ped. 1465, 1449).

The sex chromosomes of the two other Carmelita females, 1342-1 and -2, were also identified as *WY* by crossing them with Jamapa  $X_{sp} Y_{sr}$  males (ped. 1380 a and b).

#### Río Grijalva

Information on the sex chromosomes of the Río Grijalva platyfish population is based upon two females and four males. Although certain critical crosses were not performed, and in several crosses few offspring were obtained, the data demonstrate that both *W* and *X* chromosomes were present in these fish (Table 19). Another difficulty in interpreting the Grijalva data is that none of the fish were testcrossed with the reference stocks during the first generations in the laboratory.

The offspring of female Gp-1, which was gravid when collected, consisted of *Sp* and wild type males and females (ped. 450). One female, 450-1, was mated to a  $X_+ Y_{sa}$  male and gave rise to *Sd* males and females and wild type males (ped. 512). The absence of wild type females cannot be explained, but the appearance of *Sd* offspring among both sexes strongly suggests that the female parent possessed a *W* chromosome inherited from Gp-1. That 450-1 had the *WY* constitution was demonstrated by the fact

that one of her *Sd* sons was a *YY* male; when mated to a *XX* female heterozygous for the *Sp* gene, he sired only male offspring of the four pigment phenotypes (ped. 605).

Other evidence indicates that female Gp-1 had the *WX* constitution. After having been isolated from males for several months, she was mated with Gp-12, a male known to be *YY* (ped. 451 b), heterozygous for *Sd* (ped. 477). This mating produced both *Sd* and wild type males and females, a pattern of inheritance only possible if the mother is *WY* or *WX* and the father *YY*. One of the *Sd* males of pedigree 477 was then mated with a female known to be *XX* and, with a single exception, wild type females and *Sd* males were obtained (ped. 511). Since Gp-12 possessed two *Y* chromosomes, his male offspring must have inherited the *X* chromosome from Gp-1. Further evidence that Gp-1 possessed an *X* chromosome was obtained through additional crosses. Two *Sp* females of pedigree 450 were mated with Grijalva males Gp-14 and -15 (ped. 475, 476). Since the wild type daughters of these crosses both proved to be *XX* females (see ped. 511, 518), it follows that the *Sp* females of pedigree 450 had the *XX* constitution, and that Gp-1 must have possessed one *X* chromosome. Moreover, at least one of the males that fertilized Gp-1 in nature must have possessed the *XY* constitution, and Gp-14 and Gp-15 must be *XY* males.

When female Gp-2 was mated first with Gp-12 and then with Gp-13, exclusively male offspring were produced. This indicated that both males had the *YY* and Gp-2 the *XX* constitution.

Río Coatzacoalcos

Gordon (1951 a and b, 1954) briefly reported that platyfish collected in the Río Coatzacoalcos had the *XX-XY* type of sex-determination, but he published only the results of two crosses (ped. 270 and 274). The latter, which arose from the mating of a *WY* female (strain "Bh", of unknown geographic origin) and Cp-18, a Coatzacoalcos male, is especially noteworthy, since the entire *WY* class differentiated into functional males contrary to expectations. These results have given rise to the theory that the *Y* chromosome of these Coatzacoalcos fish is stronger "male determining" than the *Y* chromosome of other populations. This pedigree and others involving ten wild-caught Cp fish have been listed in Table 20. The macromelanophore pigment pattern of all four wild-caught females was inherited by one-half of their male and female offspring, while the pigment pattern of six wild-caught males was inherited either by all the daughters or by all the sons. This type of inheritance is characteristic of the *XX-XY* mechanism.

TABLE 13. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE DESCENDANTS OF WILD-CAUGHT *Xiphophorus maculatus* FROM LAKE PETÉN (1954 collection)

Ped. No.		Parents			Offspring									
		Female		Male	Females					Males				
					<i>Sd</i>	<i>Sp</i>	<i>N</i>	+	<i>Sd</i>	<i>Sp</i>	<i>N</i>	<i>Sd</i>	+	
532	Jp	$X_{Sp} X_{Sd}$	Pp-12	$X_+ Y_+$	—	33	—	23 <sup>1</sup>	—	49	—	—	54 <sup>1</sup>	
550	Cp <sup>2</sup>	$X_+ X_N$	Pp-13	$X_+ Y_+$	—	—	—	22 <sup>3</sup>	—	—	—	—	18 <sup>3</sup>	
545	Cp <sup>2</sup>	$X_+ X_N$	Pp-14	$Y_+ Y_+$	—	none	—	—	—	—	7	—	12	
549	Cp	$X_+ X_{Sd}$	Pp-15	$X_+ Y_+$	5	—	—	6	14	—	—	—	8	
548	Pp-2	?	Pp-11	$X_+ Y_+$	—	—	—	3	—	—	—	—	9	
592 <sup>3</sup>	548-1	?	532	$X_{Sp} Y_+$	—	—	—	8	—	—	—	—	12	
533	Pp-1	$W_+ X_+$	?	$XY, YY$	11	—	—	21	7	—	—	—	20	
626	533-1	$W_+ ?$	530-11 <sup>4</sup>	$Y_{Sd} Y_{Sr}$	9	—	—	13	13	—	—	—	8	
640	533-9	$W_+ ?_{Sd}$	564-11 <sup>4</sup>	$Y_N Y_{Sd}$	5	—	3	19	15	—	—	11	2	
574	533-3	$W_+ Y_+$	533-13	$? Y_{Sd}$	4	—	—	6	8	—	—	—	7	
602	533-5	$W_+ Y_+$	533-15	$Y_+ ?_{Sd}$	13	—	—	16	15	—	—	—	15	
595	533-8	$X_+ X_{Sd}$	548-13	$X_+ Y_+$	2	—	—	15	7	—	—	—	15	
698	595-3	$X_+ X_+$	574-13	$Y_+ Y_{Sd}$	—	none	—	—	28	—	—	—	27	
654	595-1	$X_+ X_{Sd}$	533-19	$X_+ Y_+$	4	—	—	26	3	—	—	—	16	
781	654-9	$X_+ X_{Sd}$	602-11	$Y_+ Y_{Sd}$	—	none	—	—	13	—	—	—	18	

<sup>1</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.

<sup>2</sup> The descendants of pedigree 300; see Table 20.

<sup>3</sup> Fish not scored for pigment patterns.

<sup>4</sup> Descendants of New River fish; for history of pedigree see Table 7; pedigree 626 not scored for *Sr* pattern.

TABLE 14. INHERITANCE OF PIGMENT PATTERN AND SEX RATIO AMONG THE DESCENDANTS OF SEVEN WILD-CAUGHT MALE *Xiphophorus maculatus* FROM LAKE PETÉN (1963 collection)

Parents				Offspring										
Ped. No.	Female	Male		Females				Males						
				<i>Sp</i>	<i>Sd</i>	<i>N</i>	<i>Sr</i>	<i>Sp</i>	+	<i>Sp</i>	<i>Sd</i>	<i>Sd</i>	+	
1388a	Jp	$X_{Sp} X_{Sp}$	1341-12	$Y_+$	$Y_+$	—	—	none	—	—	31	—	—	2 <sup>1</sup>
1388b	Gp	$X_{Sp} X_{Sd}$	1341-12	$Y_+$	$Y_+$	—	—	none	—	—	18	17	—	6 <sup>1</sup>
1396a	Jp	$X_{Sd} X_{Sd}$	1341-13	$X_+$	$Y_+$	—	—	—	—	35 <sup>2</sup>	—	—	—	37 <sup>2</sup>
1396b	Gp	$X_{Sp} X_{Sd}$	1341-13	$X_+$	$Y_+$	10	9	—	—	8 <sup>1</sup>	5	5	—	4 <sup>1</sup>
1437	Jp	$X_{Sd} X_{Sd}$	1341-14	$X_N$	$Y_+$	—	—	18 <sup>2</sup>	—	—	—	—	—	17 <sup>2</sup>
1408	Gp	$X_{Sp} X_{Sd}$	1341-15	$X_+$	$Y_+$	14	8	—	—	4 <sup>1</sup>	14	6	—	3 <sup>1</sup>
1413	Jp	$X_{Sp} X_{Sp}$	1341-16	$X_+$	$Y_{Sd}$	12	—	—	—	—	6	—	22	—
1580	Jp	$X_{Sr} X_{Sr}$	1413-11	$X_{Sp}$	$X_+$	—	—	—	34	19	—	—	none	—
1595	Jp	$X_{Sr} X_{Sr}$	1413-12	$X_{Sp}$	$X_+$	—	—	—	33	29	—	—	none	—
1416	Jp	$X_{Sd} X_{Sd}$	1341-17	$Y_+$	$Y_+$	—	—	none	—	—	—	—	—	55 <sup>2</sup>
1439	Jp	$X_{Sp} X_{Sp}$	1341-18	$Y_+$	$Y_+$	—	—	none	—	—	50	—	—	—

<sup>1</sup> Expressivity of pigment patterns is highly variable in all Petén hybrids. The wild type fish are undoubtedly due to nonpenetrance of the macromelanophore genes involved.

<sup>2</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.

DISCUSSION

Geography

These experiments provide convincing evidence that platyfish, *Xiphophorus maculatus*, with the *XX-XY* and *WY-YY* sex-determining mechanisms are not isolated from each other, but occur side by side and interbreed over a vast area (Table 21). By means of appropriate crosses both *W* and *X* chromosomes have been found in fishes from the Río Grijalva at Villahermosa, from the Río Usumacinta system at Sebol and Carmelita, from Lake Petén and from two areas in the Río Hondo (Table 21). The distance by air from Villahermosa (Río Grijalva) southeastward to Sebol is 400 km., from Villahermosa eastward to the mouth of the Río Hondo is 480 km., and from Carmelita south to Sebol is 180 km. This area comprises about 60 per cent. of the total range of this species. Populations in which only the *WY-YY* mechanism has been identified inhabit the New River and Belize River in British Honduras. The area in which the *WY-YY* system may exist exclusively is therefore quite limited and could extend from the mouth of the New River south for at least 210 km. to Mango Creek, the southernmost location in British Honduras from which platyfish have been taken. This is no more than 10 per cent. of the range of *X. maculatus*, since the coastal plain in British Honduras is quite narrow, varying from a width of 45 km. between the New River and the Caribbean in the north to less than 30 km. between the Maya Mountains and the sea in the

south. Only the *XX-XY* mechanism is known from the platyfish populations of the Río Jamapa, Río Papaloapan and Río Coatzacoalcos. Between the Río Grijalva and the Río Jamapa, a distance of 320 km., the coastal plain is nowhere more than 100 km. wide. This area constitutes roughly 30 per cent. of the platyfish range.

An experiment of this type is subject to a large sampling error, since *X. maculatus* as well as many other poeciliid fishes, although widespread, often exist in small, local breeding populations with only limited gene flow between them (Darnell, 1962; Gordon & Gordon, 1957; Haskins, Haskins, McLaughlin & Hewitt, 1961; Kallman, 1964). Platyfishes tend to stay close to banks, aggregate in favorable locations and apparently do not disperse over any great distances. Fish from a given collecting station are much more similar in genetic makeup to each other than to fish taken a few hundred meters or more away and may not be representative of the population of an entire stream system. The *W* or *X* chromosome or one type of male may easily be excluded by chance from a small sample. Since many crosses are often necessary for the unequivocal identification of the sex chromosomes of wild-caught females, only a few fish from each collecting station can be tested in the laboratory. The danger of accidentally excluding fish of one genotype from the analysis is thus quite serious. This difficulty is well illustrated by the platyfish from Carmelita: among the 12 fish analyzed, only one





TABLE 15. INHERITANCE OF PIGMENT PATTERN AND SEX RATIO AMONG THE DESCENDANTS OF SEVEN WILD-CAUGHT FEMALE *Xiphophorus maculatus* FROM LAKE PETÉN (1963 collection) (Continued)

Ped. No.	Parents		Male	Offspring											
	Female	Male		Females			Males								
1670	Jp	1496b-11	X <sub>sr</sub> X <sub>sr</sub>	X + Y <sub>N</sub>	Sd	Sp	Sr	+	Sp	Sp	Sd	Sr	N	N	+
1513a <sup>3</sup>	1404b-2	Jp	W + X <sub>N</sub>	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1513b <sup>3</sup>	1404b-3	Jp	W + X <sub>N</sub>	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1369	1341-5	unknown	W + ?	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1516 <sup>3</sup>	1369-1	Jp	W + Y +	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1559a <sup>1</sup>	1369-2	Jp	W + Y +	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1559b	1369-3	Jp	W + Y +	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1447	1341-8	Jp	W + Y +	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—
1451	1341-9	Jp	W + Y +	X <sub>sd</sub> Y <sub>sr</sub>	—	—	—	—	—	—	—	—	—	—	—

<sup>1</sup> In these fish the macromelanophore patterns were very weakly expressed. The wild-type fish are undoubtedly due to nonpenetrance of the pigment genes.

<sup>2</sup> Presumably N Sp, but a strong N expression masks Sp. See also ped. 1685.

<sup>3</sup> Jamapa Sd is usually not expressed in these pedigrees.

<sup>4</sup> The Nigra pattern in their N Sr brothers was very weakly developed and often did not appear until the fish were 6 months old or older. The Sr males are presumably N Sr. When these males were mated with Jp 163 A females, stripe-sided females and Nigra males were obtained.

X chromosome was found. In the sample from the Río Hondo (1963), one X chromosome was present among 13 fish (Table 21). On the other hand, in the 1954 collection from the Río Hondo the percentage of X chromosomes was much higher. Among the Sebol platyfish, four out of six females possessed the WY genotype yet all seven males tested were XY.

Because few individuals were tested, one cannot be certain that in the Belize and New Rivers only the WY-YY system occurs. It would be surprising if eventually the X chromosome were not demonstrated in the Belize River, since one of its headwaters, the Río Mopan, comes within three kilometers of tributaries to the Río Hondo and to streams running into Lake Petén. No obvious physical boundaries separate the stream systems from each other.

The evidence for the XX-XY system of the Río Jamapa populations is based upon experiments involving 11 fish (Gordon, 1947), part of a larger collection taken at the very mouth of the river. The claim for the XX-XY mechanism of the Río Papaloapan population rests upon a still smaller sample. The crosses reported by Gordon (1947) do not involve wild-caught fish but their descendants. All eight fishes listed in his table may have been the offspring of a single mating. This is certainly true for all Sb (Spotted-belly) fish, since this gene can be traced back to one Sb male (Gordon, 1946). The analysis of the Coatzacoalcos population is based upon ten fish. Nothing is known about the sex-determining mechanism of the fish from the Río Tonalá. Since numerous swamps and water-courses connect this river with the Río Grijalva, both W and X chromosomes would be expected here, too.

Stability of Sex-Determining Mechanism

The sex-determining mechanism of *X. maculatus* is a stable one. A 1:1 sex ratio has been observed in our laboratory stocks, some of which have been inbred at least 30-42 generations. Similarly, Bellamy & Queal (1951) found no significant deviation from a 1:1 ratio among the offspring of several hundred crosses involving domesticated strains of platyfish. There are four types of matings (WY ♀ × YY ♂, WY ♀ × XY ♂, WX ♀ × YY ♂ and XX ♀ × XY ♂) that nearly always give rise to a 1:1 sex ratio, regardless of whether the parents belong to the same or to different geographical populations (Table 22). A ratio of three females to one male is obtained from crosses between WX ♀ and XY ♂ (Table 22), and YY males always sire all-male broods when mated with XX females (Table 23).

TABLE 16. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF FIVE WILD-CAUGHT MALE *Xiphophorus maculatus* FROM THE RÍO DE LA PASIÓN (SEBOL)

		Parents		Offspring									
		Female		Male		Females				Males			
Ped. No.	(Reference Strain)	(Wild-caught)											
						Sd				Sd			
						Sd	Sp	Sp	+	Sd	Sp	Sp	+
1347a	Jp	$X_{Sp} X_{Sp}$	1328-12	$X_{Sd} Y_+$	—	—	35	—	—	32	—	—	
1347b	Gp	$X_{Sp} X_{Sd}$	1328-12	$X_{Sd} Y_+$	6	—	9	—	4	9	1	4 <sup>1</sup>	
1345a	Jp	$X_{Sd} X_{Sd}$	1328-11	$X_+ Y_{Sp}$	—	—	—	38 <sup>2</sup>	—	44 <sup>2</sup>	—	—	
1345b	Gp	$X_{Sp} X_{Sd}$	1328-11	$X_+ Y_{Sp}$	4	8	—	11 <sup>1</sup>	—	13 <sup>1</sup>	6	3 <sup>1</sup>	
1359a	Jp	$X_{Sp} X_{Sp}$	1327-13	$X_+ Y_+$	—	18	—	—	—	20	—	—	
1359b	Gp	$X_{Sp} X_{Sd}$	1327-13	$X_+ Y_+$	10	12	—	—	17	14	—	—	
1362a	Jp	$X_{Sp} X_{Sp}$	1327-14	$X_+ Y_+$	—	39	—	—	—	16	—	—	
1362b	Gp	$X_{Sp} X_{Sd}$	1327-14	$X_+ Y_+$	4	14	—	3 <sup>1</sup>	6	6	—	3 <sup>1</sup>	
1493	Hp-2	$X_+ X_+$	1328-14	$X_{Sd} Y_+$	12	—	—	—	—	—	—	15	
Total Number:					223				213				

<sup>1</sup> These fish were sacrificed at the age of three months before all pigment patterns were fully developed.

<sup>2</sup> Nonpenetrance of Jamapa Sd in inter-river hybrids.

One exceptional situation has been found. In 18 of the 20 crosses between *WY* females of the Río Hondo (the Douglas and Tikal locations) and *XY* males, females outnumbered males. The excess of females was statistically significant in only one pedigree, but when the offspring of all 20 crosses are grouped, the deviation from 1:1 becomes highly significant (Table 22). Crosses between Río Hondo males and females were not made in sufficient numbers to ascertain whether a high percentage of females is characteristic for the population or whether this occurs only in certain inter-population matings. However, no deviation from a 1:1 sex ratio has been observed in the two inbred Río Hondo strains. Differential mortality after birth cannot explain the excess of females, since the number of fish that died before sexual maturity is negligible. The preponderance of females may result from a selective mortality of males before birth (although this cannot be the case in the other stocks or populations that exhibit a 1:1 sex ratio), from a preferential fertilization of "*W*" eggs, or from a not quite random segregation of chromosomes during oogenesis so that slightly more *W* than *Y* chromosomes are incorporated into the developing egg.

The slight excess of males produced in crosses of the type  $XX \text{ ♀} \times XY \text{ ♂}$  that involve Petén fish can mainly be attributed to two pedigrees. In addition, eight other males are known to be genetic sex reversals, that is, *XX* males (Table 25).

Platyfish with the *XY* constitution always dif-

ferentiate into males regardless of the population from which the chromosomes have been derived; when mated with *XX* females from their own or from different populations, 66 males (*YY*), all but two of which were wild-caught, sired a total of 3,479 young, all of which were males (Table 23). But when ten of the same males were mated with *WY* females, their offspring were of both sexes and of equal frequency.

#### Crossing Over between Sex Chromosomes

In many matings, the sex chromosomes of the parents were marked by pigment genes so that specific patterns were restricted to one sex. Phenotypic exceptions represent either crossovers or sex reversals. Without additional crosses, however, these cannot be distinguished. In four cases, nevertheless, fish showing color patterns of the opposite sex could be classified as sex reversals without further matings (see footnote,<sup>2</sup> Table 25).

Crossing over between *W* and *Y* chromosomes was demonstrated in two out of 1,334 fish (0.2%). This value is nearly identical with the frequency of crossing over between the *X* and *Y* chromosomes (Table 24). Nine out of 22 exceptions among 5,136 *XX* or *XY* offspring were so identified (0.2%). If half of the untested exceptions are also considered crossovers, the frequency is raised to about 0.3 per cent. This rate is similar to the one reported by Bellamy & Queal (1951) for their domesticated platyfish stocks. These authors found that roughly 0.5 per cent. of their fish were exceptions of which about one-half were crossovers



and the other half sex reversals. These values are lower than those of Gordon (1937 a), who estimated the frequency of crossing over between the *W* and *Y* (= *Z*) chromosomes to be 1 per cent.

Crossing over between heterochromosomes has also been found in the egg-laying cyprinodont, *Oryzias latipes*, which has an *XX-XY* mechanism. Yamamoto (1964) reports that in normal males, the incidence of crossing over between pigment gene *R* and the sex-differential locus or segment is 0.2 per cent., while in *XY* females (produced by treating newly hatched *XY* fish with estrone) crossing over occurs in 1 per cent. of all cases.

In spite of the rarity of crossing over, in at least one brood there were two cases (ped. 1498 a, Table 24). In two pedigrees crossovers were accompanied by other exceptional fish (Table 24). Unfortunately they were not tested and their genotypes are not known. If they were crossovers, some sort of genetic factor may be present that greatly increases the frequency of crossing over. Such a factor is perhaps operative in the Petén fish; an unexpectedly high number of crossovers occurred among their descendants (among the 602 offspring of 9 *XY* Petén males there were five crossovers). If the exceptional sibs of the crossovers were sex reversals, however, the events would appear to be related, since sex reversals are also very rare. In this connection, the statement by Bellamy & Queal (1951) that crossover *WY* females give rise to an increased number of exceptional *WY* males is significant. Unfortunately, these investigators did not publish their complete data. Perhaps under certain circumstances crossing over involves in part the sex differential segment of the sex chromosome.

#### Sex Reversal

All the fish in Table 25 possessed pigment patterns that permitted them to be assigned a specific sex chromosome constitution. Of the exceptional individuals, 103 are considered without question to be sex reversed, that is, to have the phenotype of one sex and the genotype of the other. The genotypes of 17 fish remain unidentified, and these are the same 17 untested exceptions that were listed in Table 24.

The incidence of sex reversals in our matings is slightly more than 1 per cent. (Table 25). The data, however, are strongly biased in favor of the exceptions. Not only were many found among the highly inbred Jamapa strains, but broods of Jp 163 A and B in which no exceptions occurred were not necessarily recorded, while all broods with sex reversals were, of

course, counted. Moreover, many *XX* males were the result of the selective mating of sex-reversed fish. If the inbred Jamapa fish and the offspring of sex reversals (ped. 1580 and 1595 in Table 14; ped. 1685, 1570, 1686, 1687 and 1724 in Table 15) are omitted from Table 25, one may obtain a better estimate of the frequency of sex reversal. The number of *XX* females and males then becomes 1,781 and 8, respectively, and the number of *XY* males and females 5,404 and 4, respectively. With this correction, the number of sex reversals is 41 (4 *XY* ♀, 8 *XX* ♂, 29 *WY* ♂), 36 of which occurred in three pedigrees. The total frequency of sex reversal then becomes 0.5%.

All 30 *XX* males can be traced back to two fish. Petén male 1341-16 gave rise to six exceptions in a single brood, and Petén female 1341-1 was the progenitor of the remaining 24 (Table 15). Since both Petén fish were collected in the same seine haul, they may be closely related and perhaps all *XX* males have descended from a single fish.

Of the 44 *XY* females, 40 were discovered among the inbred Jamapa strains. Again, the appearance of exceptional fish is not a random event, since five females gave rise to 36 sex reversals (Table 5). Three *XY* females occurred in the same brood of Grijalva fish (Table 3). All but two of the 29 *WY* males occurred in Gordon's (1951 a) cross in which the entire *WY* class differentiated into females (ped. 274, Table 20). Of the 17 unexplained exceptions, 13 occurred in six pedigrees associated with crossovers or other exceptional individuals. Sex reversals are thus not isolated events, but are definitely concentrated in certain pedigrees.

Although the phenomenon of sex reversal has been demonstrated and studied extensively in at least three species of cyprinodont fishes, the medaka, *Oryzias latipes*, the guppy, *Poecilia reticulata*, and *Xiphophorus maculatus*, it is still not well understood. Almost all of the explanations offered to account for sex reversals in these fishes can be considered as a variation of an idea that originated with Winge (1934). He suggested that in the guppy there are many genes on the autosomes working either in a male or female direction and that in rare cases, perhaps through crossing over, many "potent" genes favoring one sex become located in one autosome and that such an autosome then has a disproportionate effect on sex-determination. Yamamoto (1963), in his discussion of spontaneous sex reversals in the medaka, states that the ratio of the sum of male- to the sum of female-determining genes on the autosomes of a population has a mean around which varia-



TABLE 17. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF SIX WILD-CAUGHT FEMALE *Xiphophorus maculatus* FROM THE RÍO DE LA PASIÓN (SEBOL) (Continued)

Ped.	Parents		Offspring														
	Female	Male	Females				Males				Males						
			Sd	Sp	Sr	Sd	Sp	Sr	Sd	Sp	Sr	Sd	Sp	Sr	Sd	Sp	Sr
1560	Hp-2	X <sup>+</sup> X <sup>+</sup>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		W <sup>+</sup> Y <sup>+</sup>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1517a	1375-1	W <sup>+</sup> X <sup>+</sup>	—	11	9	—	—	—	—	—	—	—	—	—	—	—	—
1517b	1375-2	W <sup>+</sup> X <sup>+</sup>	—	29	10	—	—	—	—	—	—	—	—	—	—	—	—
1525	1375-3	W <sup>+</sup> X <sup>+</sup>	—	23	8	—	—	—	—	—	—	—	—	—	—	—	—

<sup>1</sup> Nonpenetrance of Jamapa Sd in inter-river hybrids.

<sup>2</sup> The males were uniformly spotted. The Sp and Sr patterns could not be distinguished from each other.

tion takes the form of a normal distribution curve. According to Yamamoto, the Y chromosome shifts this ratio decidedly toward the male and the X toward the female. The rare sex reversals are those individual variants at the extreme right or left of such a curve. Similarly, Anders & Anders (1963) and Öktay (1959 a, b) attribute the occurrence of XY females or XX males in the platyfish to the effects of autosomal sex genes. They visualize that on rare occasions fortuitous combinations of autosomes possessing a large number of male- or female-determining genes override the switch mechanism of the sex chromosomes. Öktay suggests that the high incidence of XX males in one of her stocks resulted from the presence of many autosomal genes with a male tendency. As she points out, some support for such a view is provided by the fact that when XX males are outcrossed to unrelated females, very few sex reversals are found among the offspring. Similar observations have been made by Aida (1936) in the medaka and by Winge (1930, 1934) and Winge & Ditlevsen (1948) in the guppy.

Yamamoto (1963) has also pointed out, however, that the medaka has 24 pairs of chromosomes and that the possible number of germ cells with different configurations of autosomes is  $2^{46}$ . If there are many autosomal genes that influence sex determination, the chance of obtaining the necessary fortuitous autosomal combinations to effect sex reversal is small. The haploid number for the platyfish is also 24 (Friedman & Gordon, 1934). The occurrence of sex reversal, therefore, should be a rare, isolated event and the offspring of a sex-reversed individual should be normal. But as our experiments have demonstrated, and also those of Anders & Anders (1963) and Öktay (1959 a, b), just the opposite is true. These observations cannot be reconciled with the theory of a large number of autosomal male and female sex genes. Öktay's original XX male was found among the first backcross generation between two unrelated platyfish stocks. We may, therefore, assume that there was considerable genetic diversity. Yet when this fish was bred, more XX males occurred. When they were mated with their sisters, a stock was established in which both sexes were characterized by the XX constitution. One would expect that after nine generations of inbreeding the percentage of males would have increased considerably and that the sex ratio would have become stabilized with the increasing homozygosity, but this was not the case. Although Aida (1936), in *Oryzias*, was able to increase the percentage of XY males through inbreeding and selection, so that in



TABLE 18. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN IN CROSSES INVOLVING WILD-CAUGHT *Xiphophorus maculatus* FROM THE RÍO SAN PEDRO DE MÁRTIR (CARMELITA)

Ped. No.	Parents				Offspring							
	Female		Male		Females				Males			
					<i>Sp</i>	<i>Sd</i>	<i>Sr</i>	+	<i>Sp</i>	<i>Sd</i>	<i>Sr</i>	+
1395a	Jp	$X_{Sp} X_{Sp}$	1342-11	$X_+ Y_+$	19	—	—	—	14	—	—	—
1395b	Gp	$X_{Sd} X_{Sp}$	1342-11	$X_+ Y_+$	9	10	—	—	5	4	—	—
1399a	Jp	$X_{Sp} X_{Sp}$	1342-12	$Y_+ Y_+$	—	none	—	—	38	—	—	—
1399b	Gp	$X_{Sd} X_{Sp}$	1342-12	$Y_+ Y_+$	—	none	—	—	28	22	—	—
1455	Jp	$X_{Sp} X_{Sp}$	1342-13	$Y_+ Y_+$	—	none	—	—	27	—	—	—
1405a <sup>1</sup>	1342-3	$W_+ Y_+$	1342-13	$Y_+ Y_+$	—	—	—	4	—	—	—	7
1405b <sup>1</sup>	1342-4	$W_+ Y_+$	1342-13	$Y_+ Y_+$	—	—	—	3	—	—	—	2
1521	Jp	$X_{Sd} X_{Sd}$	1405a-12	$Y_+ Y_+$	—	none	—	—	—	—	—	55 <sup>2</sup>
1465	1405a-2	$W_+ Y_+$	Jp	$X_{Sp} Y_{Sr}$	27	—	25	—	13	—	22	—
1514	Jp	$X_{Sd} X_{Sd}$	1405b-11	$Y_+ Y_+$	—	none	—	—	—	1	—	32 <sup>2</sup>
1449	1405b-1	$W_+ Y_+$	Jp	$X_{Sp} Y_{Sr}$	23	—	18	—	13	—	18	1 <sup>3</sup>
1445	Jp	$X_{Sp} X_{Sp}$	1342-14	$Y_+ Y_+$	—	none	—	—	32	—	—	—
1412 <sup>1</sup>	1342-5	$W_+ Y_+$	1342-14	$Y_+ Y_+$	—	—	—	7	—	—	—	6
1518	Jp	$X_{Sd} X_{Sd}$	1412-11	$Y_+ Y_+$	—	none	—	—	—	2	—	52 <sup>2</sup>
1472	Jp	$X_{Sp} X_{Sp}$	1342-15	$Y_+ Y_+$	—	none	—	—	80	—	—	—
1425a <sup>1</sup>	1342-6	$W_+ Y_+$	1342-15	$Y_+ Y_+$	—	—	—	3	—	—	—	3
1425b <sup>1</sup>	1342-7	$W_+ Y_+$	1342-15	$Y_+ Y_+$	—	—	—	4	—	—	—	11
1528	Jp	$X_{Sd} X_{Sd}$	1425a-12	$Y_+ Y_+$	—	none	—	—	—	—	—	38 <sup>2</sup>
1526	Jp	$X_{Sp} X_{Sp}$	1425b-11	$Y_+ Y_+$	—	none	—	—	31	—	—	—
1380a	1342-1	$W_+ Y_+$	Jp	$X_{Sp} Y_{Sr}$	11	—	9	—	7	—	15	—
1380b	1342-2	$W_+ Y_+$	Jp	$X_{Sp} Y_{Sr}$	14	—	17	—	13	—	8	—

<sup>1</sup> Only a few fish picked at random when 10 days old were raised to maturity. All other fish were sacrificed for another experiment.

<sup>2</sup> Jamapa *Sd* usually not expressed in inter-river crosses.

<sup>3</sup> Pigment pattern in this pedigree weakly developed; this wild-type male was sacrificed before its pigment pattern appeared.

some broods males outnumbered females significantly, neither he nor Winge (1934) were able to stabilize the percentage of males. In these strains of the medaka and the guppy, matings of different males and females of the same generation gave rise to highly variable sex ratios. These observations suggest that Aida, Winge and Öktay had selected for a gene complex that made the sex-determining mechanism of the sex chromosomes highly labile and susceptible to other, still unknown, factors. Their data certainly do not demonstrate the existence of autosomal genes that effect sex *per se*.

Rare combinations of autosomal sex genes could hardly account for the sudden appearance of broods in which the entire XY class of fish consisted of sex-reversed females, especially since no exceptions had ever occurred previously in the strains [Table 5; see also MacIntyre (1961) and Anders & Anders<sup>3</sup> (1963)]. If these XY females were indeed the result of the

accumulation of "autosomal female" genes, one would expect that under the inbreeding regime to which the Jp 163 lines had been exposed, a few exceptional females would have occurred in earlier generations.

The results of several other crosses are also in conflict with the theory of autosomal sex genes. In F<sub>1</sub> generations of three crosses between Jamapa and Petén fish, several XX males were detected. Additional XX males (in still higher frequency!) occurred in the first and second backcross generation to Jamapa fish (Table 15). This series of crosses confronts us with the paradoxical situation, that on the one hand a large number of autosomal female genes must be attributed to the Jamapa strains, since XY females occur in them, while, on the other, the occurrence of XX males in the F<sub>1</sub>, 1st and 2nd backcross generations must be attributed to autosomal male genes.<sup>4</sup>

<sup>4</sup> Aida (1936) suggested that XX males in the medaka may be the result of a lowering of the female-determining potency of the X chromosome. Conceivably this could take place through translocation or crossing over involving part of the sex differential segment. An unusual X chromosome could not be the explanation for

<sup>3</sup> It is important to note that the platyfish stock of Anders & Anders was derived from the Jamapa strains which Dr. Myron Gordon brought to Europe more than 15 years ago.

TABLE 19. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE OFFSPRING OF WILD-CAUGHT *Xiphophorus maculatus* FROM THE RÍO GRIJALVA

Ped. No.	Parents		Offspring										
	Female		Male		Females				Males				
					<i>Sp</i>	<i>Sd</i>	+	<i>Sp</i>	<i>Sd</i>	<i>Sd</i>			
450	Gp-1	$W_+ X_+$	unknown		14	—	7	10	—	—	—	—	21
512	450-1	$W_+ Y_+$	451b-11	$X_+ Y_{Sd}$	—	5	—	—	14	—	—	—	8
605	518-1	$X_+ X_{Sp}$	512-11	$Y_{Sd} Y_+$	—	none	—	2	6	2	—	—	2
477	Gp-1	$W_+ X_+$	Gp-12	$Y_{Sd} Y_+$	—	7	15	—	18	—	—	—	18
511	476-1	$X_+ X_+$	477-11	$X_+ Y_{Sd}$	—	1	35	—	23	—	—	—	—
475	450-3	$X_+ X_{Sp}$	Gp-15	$X_+ Y_+$	2	—	7	7	—	—	—	—	—
518	475-4	$X_+ X_+$	475-14	$X_{Sp} Y_+$	27	—	—	—	—	—	—	—	40
476	450-2	$X_+ X_{Sp}$	Gp-14	$X_+ Y_+$	2	—	2	1	—	—	—	—	—
451a	Gp-2	$X_+ X_{Sp}$	Gp-13	$Y_+ Y_+$	—	none	—	21	—	—	—	—	25
451b	Gp-2	$X_+ X_{Sp}$	Gp-12	$Y_+ Y_{Sd}$	—	none	—	6	2	2	—	—	2

The 27 wild type fish of pedigree 274 (Table 20), which apparently have the *WY* genotype, were first reported by Gordon (1951 a), who suggested that the “*Y*” chromosome of the Coatzacoalcos strain was stronger than the *Y*

of the other stocks and epistatic to the *W* chromosome. This explanation has been accepted by most authors, although no additional experiments to test this hypothesis were ever performed.

the sex reversals in the Petén × Jamapa crosses, however, since some *XX* males of the backcross generation had inherited both *X* chromosomes from the Jamapa strain. The occurrence of *XX* males, therefore, is not dependent upon the presence of a sex chromosome derived from the Petén fish.

Unfortunately, sex determination in these cyprinodont fishes has been treated as if it were a question of merely adding up the strength of a large number of male and female factors. With such an approach, almost any exception can be explained by juggling figures and assign-

TABLE 20. SEX RATIO AND INHERITANCE OF PIGMENT PATTERN AMONG THE DESCENDANTS OF WILD-CAUGHT *Xiphophorus maculatus* FROM THE RÍO COATZACOALCOS

Ped. No.	Parents		Offspring															
	Female		Male		Females						Males							
					<i>Sd</i>	<i>Sp</i>	<i>Sr</i>	<i>N</i>	<i>Sd</i>	<i>Sd</i>	+	<i>Sd</i>	<i>Sp</i>	<i>N</i>	<i>N</i>	<i>Sp</i>	+	
270	Jp	$X_{Sr} X_{Sr}$	Cp-11	$X_{Sd} Y_+$	46 <sup>1</sup>	—	—	—	—	—	—	—	—	—	—	—	—	54 <sup>1</sup>
272	Jp	$X_{Sp} X_{Sd}$	Cp-12	$X_+ Y_+$	—	4	—	—	—	—	5 <sup>2</sup>	—	5	—	—	—	—	3 <sup>2</sup>
—	272-1	$X_{Sd} X_+$	272-11	$X_{Sp} Y_+$	—	20 <sup>2</sup>	—	—	—	—	—	—	—	—	—	—	—	22 <sup>2</sup>
273	Jp	$X_{Sp} X_{Sd}$	Cp-13	$X_+ Y_+$	—	8	—	—	—	—	10 <sup>2</sup>	—	7	—	—	—	—	7 <sup>2</sup>
—	273-1	$X_{Sd} X_+^2$	273-11	$X_{Sp} Y_+$	—	19 <sup>2</sup>	—	—	—	—	—	1	—	—	—	—	—	13 <sup>2</sup>
269	Jp	$X_{Sr} X_{Sr}$	Cp-16	$X_+ Y_N$	—	—	72	—	—	—	—	—	—	—	50	—	—	—
333	Jp	$X_{Sr} X_{Sr}$	269-11	$X_{Sr} Y_N$	—	—	43	—	—	—	—	—	—	—	29	—	—	—
341	Jp	$X_{Sr} X_{Sr}$	333-11	$X_{Sr} Y_N$	—	—	15	—	—	—	—	—	—	—	15	—	—	—
274	Bh	$W_+ Y_{Sp}$	Cp-18	$X_+ Y_+$	—	—	—	—	—	—	23	—	59	—	—	—	—	27
275	Cp-2	$X_N X_+$	Cp-15	$X_+ Y_+$	—	—	—	13	—	—	14	—	—	14	—	—	—	23
300	275-1	$X_N X_+$	275-11	$X_N Y_+$	—	—	—	20	—	—	—	—	—	7	—	—	—	11
298	Cp-3	$X_{Sd} X_+$	unknown		11	—	—	—	—	—	12	11	—	—	—	—	—	11
326	Cp-6	$X_{Sp} X_+$	unknown		9	—	—	—	—	3	—	3	—	—	—	—	—	7
391	326-1	$X_{Sd} X_+$	300-11	$X_N Y_+$	—	—	—	6	5	—	—	7	—	—	—	—	—	7
299	Cp-9	$X_{Sp} X_+$	unknown		12	—	—	—	—	11	—	—	12	—	—	—	—	19

<sup>1</sup> Fish sacrificed before *Sr* pattern developed.  
<sup>2</sup> Nonpenetrance of Jamapa *Sd* in inter-river hybrids.

TABLE 21. SEX CHROMOSOME CONSTITUTION OF WILD-CAUGHT *Xiphophorus maculatus*

Location	Number of Females						Number of Males	
	WW	WY	WX	W? <sup>1</sup>	XX	X? <sup>1</sup>	XY	YY
Belize River	—	4	—	—	—	—	—	2
New River	—	1	—	—	—	—	—	2
Río Hondo System								
Río Hondo, San Antonio, 1954	—	3	—	—	2	—	1	—
Río Hondo, Douglas, 1963	1	8	—	—	—	—	1	3
Aguada Corriental (Tikal)	—	8	—	—	—	—	—	6
Lake Petén								
Lake Petén, Flores, 1954	—	—	1	—	—	—	3	1
Lake Petén, Remate, 1963	—	4	1	1	—	—	4	3
Río Usumacinta System								
Río de la Pasión, Sebol	—	4	—	—	1	1	7	—
Río San Pedro de Mártir, Carmelita	—	7	—	—	—	—	1	5
Río Grijalva	—	—	1	—	1	—	2	2
Río Coatzacoalcos	—	—	—	—	4	—	6	—

<sup>1</sup> The analysis for two fish is incomplete. Critical crosses to identify the second sex chromosome were not performed.

ing arbitrary valances to chromosomes or genes. Some of the problems involved in devising a workable scheme for polygenic sex determination have been pointed out by Kosswig (1964). The experiments of Yamamoto (1953, 1955, 1958, 1959 a and b, 1962) and Dzwilllo (1962) on fishes and of Humphrey (1945, 1948) and Mikamo & Witschi (1963) on amphibians have shown that functional sex reversals can be produced artificially when the developing individual or gonad primordium is exposed to suitable agents before a critical period, presumably the limited time during which the sex chromosomes act. The *W* chromosome in amphibia and the *X* chromosome in the medaka seem not to be necessary for the production of eggs, since functional *YY* (= *ZZ*) females can be produced. The *Y* chromosome is not needed for the normal functioning of the entire male reproductive apparatus, since functional males with the *WW* (amphibia) or *XX* (fish) constitution can be obtained. As Yamamoto has pointed out, the action of the sex genes or sex chromosomes may be restricted to the critical period of sex differentiation; thereafter they have no apparent function. We have already indicated above that Öktay's, Winge's and Aida's experiments suggest selection for a gene complex that makes the sex-determining action of the sex chromosome highly labile, but other causes cannot be excluded. That sex reversals in platyfish are often not isolated events suggest that relatively few autosomal genes are involved. As new cytological methods for chromosome analysis have

become available, many gonadal and sexual abnormalities in man and in other animals have been traced to autosomal gene mutations, non-disjunction, translocation or loss of a chromosome. Sex reversal in the platyfish may be due to similar causes, but virtually nothing is known about the chromosomes of this species except that the diploid number is 48.

#### Identity of the *Y* and *Z* chromosome

Until Gordon announced that platyfish of the Río Jamapa, Río Coatzacoalcos and Río Papaloapan were homogametic in the female sex and heterogametic in the male, the sex chromosome constitution of the "domesticated" stocks of unknown geographic origin was conventionally written as *WZ* ♀ and *ZZ* ♂. In 1946 and 1947 Gordon suggested that the "*Z*" chromosome of the domesticated races might be identical with the *Y* chromosome of wild-caught Mexican fish and that the usage of *ZZ* for the domesticated male might be discontinued and *YY* substituted. In a series of intra-specific crosses involving several stocks of domesticated and wild-caught fishes, Gordon (1951 a, 1952) and Öktay (1959 a) showed that the *Z* chromosome and the *Y* chromosome were equivalent.

However, Kosswig & Öktay (1955), Öktay (1959 a and b), Zander (1962, 1964) and Anders & Anders (1963) have not only retained the symbol "*Z*" to denote the chromosome determining maleness in the domesticated races, but have also extended its use to those wild stocks in which the male is homogametic.





TABLE 23. SEX RATIOS OF MATINGS BETWEEN XX FEMALES AND YY MALES

Parents				Offspring	
Females		Males		Females	Males
Number	Strain or Population	Number	Strain or Population		
2	Jp 163; Jp 30	2	Belize River	0	304
2	Cp; Jp 163	2	New River	0	184
2	Hondo, 1954	2	Hondo, 1954	0	101
14	Cp; Jp 163 A, B; Jp 30	12	Hondo, 1963	0	648
2	Sebol	1	Hp-1	0	85
23	Gp; Jp 163 A, B; Jp 30	19	Tikal	0	903
3	Cp; Petén	3	Petén, 1954	0	105
6	Gp; Jp 163 A, B	5	Petén, 1963	0	260
8	Hp-2; Jp 163 A, B; Sebol	8	Sebol	0	381
10	Gp; Jp 163 A, B	9	Carmelita	0	438
2	Gp	3	Grijalva	0	70
74		66		0	3,479

SEX RATIOS OF MATINGS BETWEEN WY FEMALES AND SOME OF THE SAME YY MALES

					Total	
2	Np	2	Hondo, 1963	48	53	101
2	Tikal	2	Tikal	92	73	165
3	Sebol	3	Sebol	130	118	248
5	Carmelita	3	Carmelita	21	29	50
12		10		291	273	564

*X. hellerii* females and *XY maculatus* males<sup>5</sup> in which the *Y*-class hybrids consisted of 66 females and 59 males. This is a ratio indistinguishable from the sex ratio of the "Z" class  $F_1$  hybrids in some of Kosswig's (1931) earlier work, but quite different from the observations of Gordon and Rosen and Anders and Anders (Table 26). With these crosses the alleged difference between the "Z" and *Y* class hybrids disappears completely. Kosswig & Öktay (1955) minimize the importance of these findings by attributing them to variables introduced by the *X. hellerii* strain.

Zander (1964) has presented an extensive series of experiments which clearly show that the sex ratio of platyfish-swordtail hybrids depends in part upon the subspecies from which the swordtail parent was taken. He found that the number of males in the "Z" class ranged from 58 to 100 per cent., in the *Y* class from 27 to 95 per cent., and in the *X* class from 0 to 54 per cent.

In other crosses involving heterogametic platyfish females of domesticated stocks and swordtail males, all "Z" class hybrids differen-

tiated into males (Kosswig, 1928; Kosswig & Öktay, 1955; Sengün, 1941). Kosswig & Öktay attribute the absence of females among the "Z" class to cytoplasmic factors or to variables introduced by the swordtail strain. It is interesting to note that the sex ratio of the *X* class hybrids is just as variable (Table 26).

The value of hybrid data in elucidating the factors involved in sex determination in *Xiphophorus* may be seriously doubted in view of the many physiological, developmental, endocrinological, behavioral and anatomical abnormalities that have been recorded for these hybrids (Atz, 1962; Clark, Aronson & Gordon, 1954; Gordon, 1937 b, 1948; Gordon & Rosen, 1951; Kosswig, 1929; Öztan, 1960, 1963; Rosen, 1960; Sengün, 1950; Tavalga, 1949). In these hybrids, variable sex ratios and abnormal gonadal differentiation are manifestations of a general breakdown of developmental homeostasis resulting from the juxtaposition of two dissimilar genomes. Moreover, it is difficult to interpret the sex ratios of the hybrid crosses when that of *X. hellerii* itself varies greatly; in certain strains males predominate, in others females (Breider, 1935; Peters, 1964; Kosswig, 1964).

<sup>5</sup> These platyfish males are also descendants of the Jamapa fish.

At the most one can conclude (Table 26) that the sex ratios as well as the degree of gonadal differentiation of the hybrids may very well depend upon the stock of swordtails used. None of these  $F_1$  data suggest that the "Z" chromosome is different from the Y, since no difference in their genetic behavior has ever been reliably indicated.

Anders & Anders (1963) suggest that *X. maculatus* has evolved from an ancestor with a polygenic sex-determining system and that the step from the polygenic stage to one involving sex chromosomes has occurred twice, once leading to the WZ-ZZ and once to the XX-XY mechanism. According to these authors, during the polygenic stage all chromosomes carried male- and female-determining factors, but when the sex chromosome mechanism evolved, the genes working in the direction of the homogametic sex were lost from the chromosome determining the heterogametic sex. Thus female ("F") factors were eliminated from the Y chromosomes and male ("M") factors from the W, creating two strong sex-determining chromosomes. The "Z" and X chromosomes retained "M" and "F" factors.

As support for their hypothesis Anders & Anders (1963) report that YY males (produced by mating a rare XY female with a XY male) mature 3-4 weeks later than their XY brothers and suggest that the X chromosomes, in addition to carrying "F" genes, also possess genes that control the "normal" onset of sexual maturation in the male. Just how these observations provide evidence for or against the presence of "M" factors in the X chromosome is not clear. That YY (*Sr Sr*) males, which are fully fertile and functional, mature slightly later and therefore grow larger, may be a metabolic effect related to the presence of two Y chromosomes. Gordon & Gordon (1954) reported that *Sr* males of the Jamapa population were larger and relatively more deep-bodied than Jamapa males with other pigment patterns. Such an effect could be produced by a gene linked to *Sr* that influences the time of sexual maturity. In YY males homozygous for the *Sr* allele, this effect might well be accentuated. The occurrence of males with different body proportions related to the age of sexual maturation (as well as the extent to which other secondary sex characters are developed), is a characteristic feature of the genus *Xiphophorus* and other poeciliid genera (Rosen & Bailey, 1964). The polymorphism exhibited by adult males probably finds its most extreme expression in the swordtail, *Xiphophorus pygmaeus nigrensis* (Rosen, 1960).

The difference in the sex ratio of the hybrids

of *hellerii* and *maculatus* with "Z" and Y chromosomes has also been used as evidence that "F" genes are present in the "Z", but absent from the Y chromosome. As previously mentioned, however, the percentage of females in the Y class was just as high as that in the "Z" class in several crosses (Table 26). Zander's (1964) recent experiments show that the percentage of females is sometimes even higher in hybrids belonging to the Y class — just the opposite of what the theory of Anders & Anders would lead one to expect.

The occurrence of ZZ females and the absence of YY females has also been cited to support the presence of "F" genes in the "Z" chromosomes; YY females do not occur, since "F" genes are missing from these chromosomes. But the "ZZ" females to which Anders refers (Kosswig, 1931, 1936) were not *X. maculatus* females; they were hybrids between the platyfish and the swordtail.<sup>6</sup> Since all crosses involving these two species have shown that the sex chromosomes of *X. maculatus* do not manifest themselves normally in the hybrids, the data cannot be accepted as proof of the existence of "F" genes in the "Z" and their absence from the Y chromosome.

According to the theory of Anders & Anders, exceptional WZ males would be relatively rare, since the W chromosome possesses only "F" factors and the Z chromosome both "M" and "F" genes. However, exceptional WY males have been reported on several occasions (Breider, 1942; Bellamy & Queal, 1951; Öktay, 1959 a).

The simultaneous existence of W and X chromosomes and homogametic and heterogametic males in the majority of the platyfish populations and the demonstration that the male-determining chromosomes of *X. maculatus* and *X. variatus* can replace each other (Bellamy, 1936; Gordon & Smith, 1938; Kosswig, 1935; Öktay, 1959 a, 1962), is excellent evidence that the "Z" and Y are one and the same chromosome.

#### Evolution of Sex-determining Mechanism in *Xiphophorus maculatus*

Gordon (1952) thought that populations with the WY-YY and XX-XY systems were geographically isolated and suggested that each specialized mechanism arose independently from an undifferentiated polygenic condition, perhaps like the one that exists in the swordtail. Anders & Anders (1963) presented an essen-

<sup>6</sup> (*hel* ♀ × *mac* ♂) ♀ × [(*hel* ♀ × *mac* ♂) ♀ × *mac* ♂] ♂



TABLE 24. CROSSING OVER BETWEEN THE SEX CHROMOSOMES OF *Xiphophorus maculatus*

Stock of Fish	Between W and Y		Between X and Y	
	Total Number of Fish	Number of males and females showing pigmentation of opposite sex	Total Number of Fish	Number of males and females showing pigmentation of opposite sex
Np (Table 2)	232 <sup>1</sup>	2 1 ♀ is x-over (ped. 1200, page 155); 1 ♀ not tested <sup>2</sup>	614	2 2 ♂ not tested
Hp-1 (page 155)	125	0	437	5 1 ♂ ped. 864 is x-over; 1 ♂ ped. 718 is x-over (page 156), 2 ♀ & 1 ♂ of ped. 718 not tested.
Belize (Table 6)	114	0	425	1 1 ♂ not tested
New River (Table 7)	172	1 1 ♂ not tested	1,494	2 2 ♂ are x-overs, ped. 1297, 1700 (page 158)
Río Hondo (Table 8)	44	0	58	0
Río Hondo (Table 10)	252	3 1 ♂ ped. 1461 not tested, but a sib was sex-reversal; 2 ♀ ped. 1555 not tested	16	0
Tikal (Table 12)	168	0	75	0
Sebol (Table 17)	227	1 1 ♀ ped. 1421 is x-over	1,023	10 1 ♀ ped. 1485 is x-over; 2 ♂ & 2 ♀ ped. 1649 not tested; 1 ♀ ped. 1504 is x-over, 1 ♀ not tested; 1 ♀ & 1 ♂ ped. 1498a are x-overs; 1 ♂ ped. 1496 is x-over
			205	1 1 ♂ ped. 1347 not tested
			239	0
			126	1 1 ♀ ped. 511 not tested

TABLE 24. CROSSING OVER BETWEEN THE SEX CHROMOSOMES OF *Xiphophorus maculatus* (Continued)

Stock of Fish	Between <i>W</i> and <i>Y</i>		Between <i>X</i> and <i>Y</i>	
	Total Number of Fish	Number of males and females showing pigmentation of opposite sex	Stock of Fish	Number of males and females showing pigmentation of opposite sex
			Río Coatzacoalcos (Table 20)	
Number of offspring:	1,334			424
Verified cases of crossing over:	2			0
Sex-reversals or crossing over:	5			
				5,136
				9
				13

<sup>1</sup> The wild type females as well as all males listed in Table 2 have not been used in this tabulation, since the great variation in the expression of macromelanophore patterns makes the discovery of crossovers difficult.

<sup>2</sup> This female is undoubtedly a crossover. If it were a sex-reversal, it would be the first known case of a *YY* female.

tially similar view which has already been discussed in the previous section. This view implies that sex chromosomes did not develop in the platyfish until the last million years and that during most of the 350 million years that fishes have existed the multitude of forms ancestral to *Xiphophorus* and the poeciliids possessed no sex chromosome mechanism. This seems most unlikely, since sex chromosomes are widespread elsewhere. The platyfishes possess 24 pairs of chromosomes and it would be a remarkable coincidence, indeed, if the same pair had independently evolved into sex chromosomes not only in the two hypothetical isolated platyfish populations, but also in *X. variatus*, the sex chromosomes of which are homologous to *W*, *X* and *Y* of *maculatus* (Atz, 1959; Öktyay, 1962). Moreover, the geography of the area does not provide any evidence that such a separation ever existed in the past. The coastal plains of southern Mexico and British Honduras are continuous across the Petén district of Guatemala. The large interior lakes and swamps of northern Petén provide ready-made avenues of dispersal between the Caribbean lowlands to the East and the Gulf of Mexico coastal plain to the West.

Rosen (1960) suggests that *X. maculatus* or its immediate ancestor invaded its present range from an area in northern Veracruz and southern San Luis Potosi where the genus *Xiphophorus* seems to have originated. No significant differences in morphological traits between platyfish inhabiting each of the river systems have developed (Gordon & Gordon, 1954; Rosen, 1960), although the frequency of the macromelanophore alleles and tailspot patterns is different for every drainage (Gordon & Gordon, 1957). During the Pleistocene, the ocean level may have been sufficiently low to permit the movement of platyfish between river systems (Rosen, 1960). The difference in the frequency of the pigment patterns would then be of more recent origin.

The *W* and *X* chromosomes are found together in the center of distribution of *X. maculatus* in the Río Grijalva and Río Usumacinta drainage. These rivers are contiguous near their mouths and form the largest river system in Central America. Both chromosomes are also found in Lake Petén and Río Hondo. The *W* seems to be absent from the Río Coatzacoalcos, Río Papaloapan and Río Jamapa to the West and the *X* may be missing from the New and Belize Rivers at the eastern edge of platyfish distribution. As more populations are examined, this picture may change, but present evidence thus indicates that the *W* chromosome has





TABLE 26. SEX RATIO OF F<sub>1</sub> HYBRIDS BETWEEN *Xiphophorus maculatus* AND *Xiphophorus hellerii*

Refer- ence*	Class:										Refer- ence*
	Xx		Yx		Zx		Wx				
	♀	♂	Other	♀	♂	♀	♂	♀	♂	♀	
a	100% <sup>3</sup>	—	<i>xx (hel.)</i> ♀ <sup>1</sup> × <i>XY (mac.)</i> ♂ :	6%	4%	<i>xx (hel.)</i> ♀ × <i>ZZ (mac.)</i> ♂ <sup>2</sup> :	11	14	e		
b <sup>4</sup>	102	18		66	59		164	484	f <sup>6</sup>		
c	♀ and ♂	in varying ratios		2	—	90% sterile males	0	16	d <sup>5</sup>		
d <sup>5</sup>	16	19		42	40	all others sterile	8	92	d <sup>7</sup>		
d <sup>7</sup>	91	8		42	81		135	200	d <sup>8</sup>		
d <sup>8</sup>	293	12		189	97						
c	40	4	<i>XY (mac.)</i> ♀ <sup>9</sup> × <i>xx (hel.)</i> ♂ :	0	—	29—sterile males, 15—male differentiation barely indicated; 15—undifferentiated	0	10	5	0	g
i	94 <sup>10</sup>	10 <sup>10</sup>	<i>XX (mac.)</i> ♀ × <i>xx (hel.)</i> ♂ :	—	—		0	36	48	0	h
c	< 50%	< 50% <sup>11</sup>		—	—		0	26	21	1	b
c <sup>12</sup>	86	15		—	—		0	—	—	—	—
d <sup>13</sup>	127	2		—	—		—	—	—	—	—
d <sup>7</sup>	199	2		—	—		—	—	—	—	—
d <sup>8</sup>	276	105		—	—		—	—	—	—	—

\* a: Gordon & Rosen, 1951; b: Kosswig & Öktay, 1955; c: Anders & Anders, 1963; d: Zander, 1964; e: Bellamy, 1922; f: Kosswig, 1939; g: Kosswig, 1928; h: Sengün, 1941; i: Berg & Gordon, 1953.

<sup>1</sup> Since no sex chromosomes of *X. hellerii* are known, the chromosomes homologous to the X, W and Y of *X. maculatus* are conventionally written as "xx".

<sup>2</sup> We have used here Kosswig's symbol "Z" to denote the male determining chromosome of those *X. maculatus* strains in which the male is homogametic.

<sup>3</sup> Mostly fertile.

<sup>4</sup> A summary of several crosses.

<sup>5</sup> A summary of several crosses; parent — *X. h. hellerii*.

<sup>6</sup> A summary of a large number of crosses with widely fluctuating sex ratios, (see also Kosswig, 1928, 1931, 1934). Some of these fish were sterile or only partially differentiated and others exhibited greatly delayed maturity. A complete breakdown is not available.

<sup>7</sup> A summary of several crosses; parent — *X. h. strigatus*.

<sup>8</sup> A summary of several crosses; parent — *X. h. guntheri*.

<sup>9</sup> An exceptional XY female.

<sup>10</sup> All fish tested proved fertile.

<sup>11</sup> A summary of several broods or crosses. Sex ratio varied considerably, but there were never more than 50% males.

<sup>12</sup> A summary of crosses involving platyfish females that were related to pedigrees in which exceptional XY females occurred.

<sup>13</sup> A single cross; female parent — *X. h. hellerii*.

is constantly active in courting and attempting to inseminate many females. One male can fertilize a large number of females, and a single insemination may be sufficient for several successive broods. There is direct evidence that in several species females greatly outnumber males. In *Gambusia* (Krumholz, 1963) and in the guppy (Haskins *et al.*, 1961), the number of males becomes drastically reduced through predation. In at least two species of *Poeciliopsis*, there are females that give rise to offspring of both sexes, but there are other females that give rise to all-female progeny and this all-female condition is inherited (Miller, 1960; Schultz & Miller, 1959; Schultz, 1961). Several of such all-female strains have been perpetuated in the laboratory for many generations. They do not reproduce by gynogenesis, since paternal traits are expressed in the offspring (Miller & Schultz, 1959; Schultz, 1961). Hubbs (1964) reports that in two populations of *Poecilia latipinna* examined, females outnumber males. Breeding experiments in the laboratory indicate that the sex ratio in favor of females has, at least in part, a genetic basis. *Poecilia formosa* is an all-female species that reproduces by gynogenesis after mating with males of closely related species (Hubbs & Hubbs, 1932; Hubbs, 1964; Kallman, 1962). An outstanding example that a 1:1 sex ratio is not necessary in poeciliids is provided by the *P. latipinna* and *P. formosa* populations of Brownsville, Texas. In this area females of *P. latipinna* and *P. formosa* outnumber males 25-100:1 (Hubbs, 1964).

In those species in which a 1:1 sex ratio is evidently not necessary, and in which an excess of females may even be advantageous, the sex-determining mechanism could undergo an adaptive radiation and evolve into new specialized systems. Not all such evolutionary experiments might be successful, but those resulting in a sex ratio favoring females would be strongly selected for. The *W* chromosome of *X. maculatus* may represent such an experiment. If this view is taken, the polygenic system of *X. hellerii* should not be considered primitive but a more specialized condition that arose from the *XX-XY* mechanism. Sex chromosomes are not terminal stages in evolution; like all other chromosomes, they evolve and change through translocations, inversions and deletions, and the function of sex determination can be taken over by other chromosomes (White, 1954). To consider the sex-determining mechanism of the swordtail as advanced is also in much better accordance with the fact that *X. hellerii* is the most widespread, ecologically diverse and specialized member of the genus (Rosen, 1960).

The *XX-XY* and the *WY-YY* systems of *X. maculatus* cannot be regarded as two distinct sex-determining mechanisms. The *W* and *X* chromosomes occur together in many populations and breeding experiments show that fish with different chromosome constitutions breed and in all ways are compatible with each other. The *W*, *X* and *Y* chromosomes of *X. maculatus* are also homologous to the *X* and *Y* chromosomes of *X. variatus*. The two species undoubtedly evolved from a common ancestor with an *XX-XY* mechanism. Most likely, the *W* arose from the *X* chromosome somewhere in the Río Usumacinta-Río Grijalva system.

#### SUMMARY

1. The platyfish, *Xiphophorus maculatus*, lives in rivers of the Atlantic coastal plain from British Honduras westwards to the Río Jamapa, Veracruz, Mexico.

2. Genetic studies during the last 35 years have shown that certain strains and populations of *X. maculatus* are heterogametic in the female, while others are heterogametic in the male sex. All domesticated stocks of unknown geographic origin and populations from British Honduras were found to possess the *WY* ♀-*YY* ♂ system, but those from the Río Jamapa, Río Coatzacoalcos and Río Papaloapan in Mexico the *XX* ♀-*XY* ♂ system.

3. Although sex chromosomes have never been identified cytologically in this species, their presence can be inferred from the behavior of a number of sex-linked pigment patterns. *X. maculatus* with different sex-determining mechanisms are morphologically indistinguishable and hybridize readily; the offspring are fully fertile. Fish with the *WY*, *WX* and *XX* constitutions differentiate nearly always into females and those that are *XY* or *YY* into males.

4. Eight stocks (6 *XX-XY*, 2 *WY-YY*) of known geographic origin have been maintained in the laboratory for many generations; one, Jp 30, inbred since 1939 for more than 42 generations by mating brother-to-sister. In all strains, males and females occur in equal numbers.

5. Platyfish were collected in all major drainage systems and their sex chromosome constitution analyzed, in order to determine the distribution of the *X* and *W* chromosomes. Fish from the New River and Belize River of British Honduras possess the *WY-YY* mechanism, but since the sample consisted of only 9 fish, the picture may change as more individuals are collected. In the Río Hondo system (British Honduras, Guatemala) *XY* (2) and *YY* (9)

males,  $XX$  (2),  $WY$  (19) and  $WW$  (1) females were collected. The fish from Lake Petén (Guatemala) consisted of  $WY$  (4) and  $WX$  (2) females and  $XY$  (7) and  $YY$  (4) males. The Río Usumacinta-Río Grijalva system, which is the largest in Central America, was sampled at three widely separated points. In the Río de la Pasión  $WY$  (4) and  $XX$  (1) females and  $XY$  (7) males were collected; in the Río San Pedro de Martir  $WY$  (7) females,  $YY$  (5) and  $XY$  (1) males and in the Río Grijalva  $WX$  and  $XX$  females (1 each) and  $XY$  and  $YY$  males (2 each) were found. No  $YY$  males and no  $W$  chromosomes are known from the Río Coatzacoalcos, Río Papaloapan and Río Jamapa. The area in which both  $W$  and  $X$  chromosomes occur together is approximately 60% of the platyfish range.

6. Fish with the various sex chromosome combinations were found in the same seine haul and breeding experiments with females that were gravid when collected indicate that  $XY$  and  $YY$  males fertilize all females, regardless of their sex chromosome constitution.

7. The sex-determining mechanism of *X. maculatus* is a stable one. When 66  $YY$  males were mated to  $XX$  females from their own or from different populations, 3,479 offspring were obtained, all males. But when 10 of the  $YY$  males were bred with  $WY$  females, offspring of both sexes were produced in equal numbers. Crosses between  $WX$  females and  $XY$  males resulted in a 3:1 sex ratio. Crosses between  $WY$  females and  $YY$  or  $XY$  males,  $WX$  females and  $YY$  males, and between  $XX$  females and  $XY$  males, regardless of whether or not the parents belonged to the same population, gave rise to a 1:1 sex ratio with the following exception. Matings between  $WY$  females of the Río Hondo system and  $XY$  males of other locations produced an excess of females.

8. Crossing over between the  $W$  and  $Y$  chromosomes in females and between the  $X$  and  $Y$  chromosomes in males occurs at a frequency of approximately 0.2 per cent.

9. The frequency of sporadic genetic sex reversals ( $WY$  ♂,  $XX$  ♂,  $XY$  ♀) is about 0.5 per cent. Sex reversals do not always occur at random, but are concentrated in certain strains and pedigrees. They may be found (a) in rigidly inbred stocks, (b) among the offspring of heterozygous parents belonging to the same or (c) to different populations. Some crosses suggest that sex reversals and crossing over may be related events. Genetic sex reversals in platyfish have been attributed to fortuitous combinations of autosomal "male" and "female" determining

genes that override the switch mechanism of the sex chromosomes. Such autosomal "sex" genes cannot account for some of the sex reversals obtained in these experiments.

10. The sex-determining mechanism of populations or strains in which males are homogametic is sometimes written  $WZ$  ♀- $ZZ$  ♂. Several authors believe that the "Z" chromosome is different from the  $Y$  of fish with the  $XX$ - $XY$  system. In intraspecific crosses no difference between the  $Y$  and "Z" chromosomes can be demonstrated. It is claimed that the difference between the  $Y$  and "Z" chromosomes becomes apparent in interspecies crosses with the swordtail, *X. hellerii*. A critical review of the *maculatus* × *hellerii* crosses, however, has failed to reveal any consistent difference between the "Z" and  $Y$  class hybrids.

11. Since the  $W$  and  $X$  chromosomes and homogametic and heterogametic males occur together over a vast area, it is best to eliminate the symbol "Z". Retaining it might lead to the misunderstanding that *X. maculatus* possesses two separate sex-determining mechanisms.

12. The  $WY$ - $WX$ - $XX$ - $XY$ - $YY$  system of *X. maculatus* is a single integrated sex-determining mechanism that arose from an ancestor which was probably  $XX$ - $XY$ . The possible evolution of the  $W$  chromosome is discussed.

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